Atomic Energy Control Board
P.O. Box 1046
Ottawa, Canada
K1P 5S9

Commission de contrôle de l'énergie atomique
C.P. 1046
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K1P 5S9

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CYTOGENETIC MEASUREMENTS OF THE RELATIVE BIOLOGICAL EFFECTIVENESS OF TRITIUM

by

C. Chopra and J.A. Heddle
Bio-mutatech Incorporated

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ABSTRACT

Chromosome aberrations in peripheral blood lymphocytes, which are used to estimate radiation dose biologically, were induced by tritium 1.14 times as effectively as X-rays (95% confidence limits: 0.8 - 1.5). Chromosome translocations in spermatogonia, which are one component of genetic risk, were induced by tritium 1.21 times as effectively as X-rays (95% confidence limits: 0.8 - 1.9). All experimental measurements were made in CBA/H mice injected with tritiated water or exposed to X-rays at a comparable dose rate.

RÉSUMÉ

Des aberrations chromosomiques dans des lymphocytes périphériques du sang, qui sont utilisées pour l'estimation biologique de la dose de la radiation, étaient induites par le tritium 1.14 fois plus effectivement que par les rayons X (95% limites de confiance: 0.8 - 1.5). Les translocations chromosomiques dans les cellules spermatogoniques, qui sont une partie du risque génétique, étaient induites par le tritium 1.21 fois plus effectivement que par les rayons X (95% limites de confiance: 0.8 - 1.9). Toutes les mesures expérimentales étaient faites sur des souris CBA/H injectées avec de l'eau tritiée ou exposées aux rayons X à des taux comparables.

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SUMMARY

Workers in a nuclear reactor facility are exposed to a variety of radiations including beta radiation from tritiated water. This is important in Canada because deuterium oxide in a CANDU reactor generates tritium. The present study was designed to evaluate the biological effectiveness of tritium relative to X-rays to provide information (1) for a cytogenetic estimate of radiation doses received by atomic energy workers and (2) for estimates of genetic hazard to these workers. Hence, two sets of measurements were made using CBA/H mice: (a) cytogenetic damage in somatic cells as seen by the induction of chromosomal aberrations in first division metaphases in peripheral blood lymphocytes (the same cells used for biological dosimetry in atomic radiation workers or radiation accidents), and (b) heritable genetic damage in germ cells as seen by the induction of translocations in primary spermatocytes.

The project consisted of two phases, a range-finding study (Phase I) to define appropriate doses for use in the definitive study for determination of the relative biological effectiveness of tritium, and a larger measurement phase (Phase II). The results of Phase I suggested that (1) the dose range should be reduced, (2) the tritium dose to individual animals should be determined because considerable animal to animal variability was observed, and (3) tritium might be more effective than X-rays. Phase II was altered accordingly.

In the Phase II study of chromosome aberrations in blood lymphocytes, female mice were treated with tritiated water or X-

rays. The RBE of tritium relative to X-rays is the ratio of the slopes of the regression lines for each effect. The RBE for dicentrics and centric rings (the aberrations used for biological dosimetry) was 1.14. In addition, Phase I showed an approximately 3- to 4-fold increase in responsiveness of splenic lymphocytes compared to peripheral blood lymphocytes for both radiations.

The Phase II translocation study utilised male CBA/H mice which were treated with tritiated water or X-rays. The RBE for translocations (rings and chains) induced by tritium was 1.21 relative to X-rays.

For each of these effects the error bars associated with the RBE are such that an RBE of 1.0, indicating an equal effectiveness of tritium and X-rays, is included within the 95% confidence interval. The lower limit of the confidence interval for each of the effects is about 0.8, whereas the upper limit varies from 1.2 in the case of terminal deletions to 2.92 in the case of interstitial deletions and acentric rings. For the aberrations of most importance for radiation dosimetry, dicentrics and rings, the upper limit of the 95% confidence interval is 1.5 indicating that biological estimation of a tritium dose is unlikely to be in error by more than 50%. For the aberrations that are significant for genetic effects, namely translocations, the upper limit of the confidence interval is 1.94. This indicates that tritium is unlikely to be as much as twice as effective as X-rays. Further work would be required for a more precise estimate.

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ABBREVIATIONS

ac	acentric ring
AI	asymmetrical interchange
BrdU	5-bromo-2'-deoxyuridine
c	centric ring
C	Celsius
Con A	concanavalin A
del	deletion
dic	dicentric
ECGS	endothelial cell growth supplement
FBS	foetal bovine serum
Gy	gray(s)
HBSS	Hank's balanced salt solution
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HTO	tritiated water
ID	interstitial deletion
inter	interchange
iso	isochromatid deletion
I.U.	international units
kVp	kilovoltage peak
LET	linear energy transfer
LPS	lipopolysaccharide
2-ME	2-mercaptoethanol
meV	millielectron volts
NA	not available
PCD	premature centromere division
PBL	peripheral blood lymphocyte

PBS	phosphate buffered saline
PI	pericentric inversion
prim.	primary animal code
p.s.i.	pounds per square inch
RBE	relative biological effectiveness
r.p.m.	revolutions per minute
RPMI	Roswell Park Memorial Institute
S.D.	standard deviation
sec.	secondary slide code
S.E.M.	standard error of the mean
SI	symmetrical interchange
SSC	saline sodium citrate
trans	translocation

GLOSSARY

Aberration:

An abnormal chromosome structure resulting from the deletion or rearrangement of chromosomal material. These may be either of the chromosome-type, which arise during the G0 or G1 phases of the cell cycle, where the whole chromosome is involved in the breakage and reunion, or of the chromatid-type, which arise during the S or G2 phases of the cell cycle, where the unit of breakage is ordinarily a single chromatid.

Acentric chromosome:

A chromosomal fragment with no centromere.

Acentric ring:

An acentric chromosome, the ends of which have joined to form a ring.

Bivalent:

The structure formed by the two homologous chromosomes when held together by one or more chiasmata during meiosis.

Centric ring:

A chromosome exchange between the two arms of a chromosome to form an acentric fragment and a ring containing a centromere.

Chain configuration:

A reciprocal translocation between two or more non-homologous chromosomes and subsequent association of homologous chromosomes at diplotene. A chain of four (C IV) is formed by the association of two bivalents where one arm lacks a chiasma; a chain of three-plus-one (C III + I) is a result of a translocation lacking chiasmata in two adjacent arms. Chain configurations of six, eight, etc. arise from multiple translocations involving one or more chromosomes in common.

Deletion:

A chromosomal fragment resulting from a break in a normal chromosome which may be either of the chromosome or chromatid (single) type.

Dicentric:

A chromosome aberration arising from an asymmetrical exchange between two chromosomes resulting in an acentric fragment and a chromosome containing two centromeres.

Gap:

A non-staining region in a chromatid arm (chromatid gap) or in the same location on both arms of the chromosome (isochromatid gap, chromosome gap) whose size is less than or equal to the width of a chromatid, and which does not cause a misalignment of the distal portion with the centromeric portion.

Interchange:

An exchange between chromatids of different chromosomes.

Interstitial deletion:

Also called double minutes, these are tiny acentric rings in which the two chromatids do not pair along their lengths but rather sit side by side like a pair of dots.

Intrachange:

An exchange of chromosomal material between chromatids of the same chromosome, or even within a single chromatid.

Inversion:

A breakage and reversal of orientation of chromosomal material. This may be either paracentric (within a chromosome arm) or pericentric (between the arms of a chromosome).

Isochromatid deletion:

A break and exchange between sister chromatids usually involving an asymmetrical union between sister chromatids either proximal or distal to the point of breakage.

Reciprocal translocation:

A chromosomal aberration in which non-homologous chromosomes exchange parts.

Ring configuration:

A structure resulting from a reciprocal translocation between two (RIV), three (RVI), or more (etc.) non-homologous chromosomes, where crossing over takes place in all arms of the bivalents.

Robertsonian translocation:

A translocation resulting in the formation of a single metacentric chromosome and a centromeric fragment resulting from an exchange between two chromosomes through their centromeres.

Univalent:

A disassociated chromosome at meiosis.

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INTRODUCTION

Workers in a nuclear reactor facility are exposed to a variety of radiations including beta rays from tritiated water. This is of particular importance in Canada because of the use of deuterium oxide as a moderator and the consequent generation of large quantities of tritiated water. Since radiations of different types and energies have different relative biological effectiveness (RBE), the RBE of tritium beta rays must be considered in the setting of permissible exposures and in the estimation of physical doses from biological effects.

The effects of high energy X-rays and gamma rays applied externally have been well documented. Although they differ somewhat for different biological effects, for most effects, and genetic effects in particular, the RBE of X- or gamma rays relative to 250 kVp X-rays is about 1.0 over a wide range, decreasing gradually to about 0.8 at energies of about 1.2 meV. Charged particles, however, can have very different RBEs and can be several times more effective than 250 kVp X-rays. These differences are due to different patterns of energy deposition, clustering of ions, and occasionally, nuclear reactions involving chemical changes. The radiation from tritium decay is a beta ray of 18.6 keV maximum energy (5.7 keV average energy). Since water is ubiquitous in biological material and enters into many biological reactions, beta rays are released not only from water within the cell, but also from tritium that is incorporated into biological molecules and the very structure of the cell itself, including the chromosomes. Knowledge of the RBE of tritium beta particles is thus important in order to estimate the oncogenic

and genetic risks from exposure to tritiated water.

Biological dosimetry, based upon the frequency of chromosome aberrations in the lymphocytes cultured from exposed or possibly exposed persons, is now a standard method of estimating external radiation dose. Acute *in vivo* doses can be estimated accurately in the range of 0.4 to 8.0 Gy by comparing the frequency of aberrations found in cultured lymphocytes with those produced by irradiation of blood *in vitro*. No such standard curve is available for beta rays from tritiated water. It is not clear whether an *in vitro* exposure of the cells to tritiated water could adequately model *in vivo* exposure in this case. Certainly no *in vitro* measurement of genetic risk is feasible. This study was designed to determine the RBE for the chromosome aberrations used for biological dosimetry, and for one component of genetic risk, translocations. Both these measurements were made on mice as models for the humans.

MATERIALS AND METHODS

A. MATERIALS

1. Chemicals

All standard chemicals used in the project were of reagent grade. All media, sera, media supplements and tissue culture products were obtained from Gibco or Flow Laboratories. Concanavalin A, lipopolysaccharide (serotype O111:B4, phenol extract) and 2-mercaptoethanol were obtained from Sigma Chemical Company. Supplemented media were prepared using aseptic technique under a laminar flow hood. All chemical solutions required to be sterile were autoclaved at 121 degrees C and 15 p.s.i. for 15-20 min.

2. Animals

CBA/H mice, obtained from Atomic Energy of Canada Limited, Chalk River, Ontario, were used for this project because the same strain was being used in a parallel study of the induction of myeloid leukemia by tritium relative to X-rays. Once received, the animals were housed in a controlled environment of 22 $\pm$ 1 degrees C and 50 $\pm$ 20% humidity and a 12-hour light/12-hour dark cycle in the Animal Care Facility of the Behavioural Sciences Building at York University. All mice received Purina Laboratory Chow #5001 and tap water *ad libitum*. Wood shavings served as bedding in polypropylene shoe box cages. The mice for Phase I were received at Bio-Mutatech Inc. on February 13, 1987 and those for Phase II (lymphocytes and spermatocytes) were received on April 23, 1987. The mice were maintained as described

above until required for the project.

B. METHODS

1. Irradiation

CBA/H mice were treated with X-rays or tritiated water at Atomic Energy of Canada Limited prior to being sent to Bio-Mutatech Inc. The following doses of X-rays and tritium were administered to mice for the different study phases:

Phase	Sex	Radiation	Dose	Date of Dosing (Date of Birth)
I lymphocytes	female	X-rays	2 or 6 Gy	Feb. 2-12, 1987 (not available)
I lymphocytes	female	HTO	2 or 6 Gy	Feb. 2, 1987 (not available)
II lymphocytes	female	X-rays	1.5, 3.0 or 4.5 Gy	April 6-15, 1987 (not available)
II lymphocytes	female	HTO	1.5, 3.0 or 4.5 Gy	April 6, 1987 (not available)
II spermatocytes	male	X-rays	1.5, 3.0 or 4.5 Gy	April 6-15, 1987 (Jan. 2-20, 1987)
II spermatocytes	male	HTO	1.5, 3.0 or 4.5 Gy	April 6, 1987 (Jan. 14-20, 1987)

Control male mice were born between January 20-26,1987.

The precise radiation dose received by each mouse based upon body weight measurements is indicated in Appendix XI. Mice were dosed with 250 kVp equivalent X-rays which were obtained from a 150 kVp filtered X-ray source (Gragtmans, personal communication). These mice were irradiated

appropriately over a 10-day period to simulate the decreasing radiation as a result of intraperitoneal injection of tritiated water. Tritium is excreted from the body such that the biological half-life is approximately 2 days. Atomic Energy of Canada Limited performed these dosings based upon this assumption. Appropriate control untreated mice were supplied for each phase.

The mice used in Phase I were mature female adults at the time of irradiation. The mice used in Phase II for the spermatocyte study were 10-13 weeks of age at the time of irradiation with dates of birth ranging between January 2-26, 1987. A total of 15 control untreated mice were received for Phase I and 8 mice were received for each treatment group. For Phase II lymphocytes, the numbers of mice received were as follows: 21 controls, 30 mice for 1.5 Gy X-ray, 15 mice each for 3.0 and 4.5 Gy X-ray, 27 mice for 1.5 Gy tritium (1 mouse died prior to shipping), and 14 mice each for 3.0 and 4.5 Gy tritium. For Phase II spermatocytes, a total of 21 control mice were received and 14 mice were received for each treatment group.

2. Study Design

a. Lymphocyte - Phase II

Dose	Number of Mice Supplied	Number of Mice Evaluated	Number of Cells to be scored per mouse	Total Number of cells scored
Tritium				
1.5 Gy	27	21 *	100	2000
3.0 Gy	14	13 *	100	944 *
4.5 Gy	14	10	50	500
X-rays				
1.5 Gy	30	20	100	2000
3.0 Gy	15	10	100	1000
4.5 Gy	15	10	50	500
Control	21	10	100	1000

b. Spermatocytes

Dose	Number of Mice Supplied	Number of Mice Evaluated	Number of Cells to be scored per mouse	Total Number of cells scored
Tritium				
1.5 Gy	14	10	200	2000
3.0 Gy	14	10	150	1500
4.5 Gy	14	14 *	100	817 *
X-rays				
1.5 Gy	14	10	200	2000
3.0 Gy	14	10	150	1500
4.5 Gy	14	10	100	1000
Control	21	15	200	3000

* A greater number of mice were evaluated because an insufficient number of cells were found on the requisite number of slides.

3. Mouse Lymphocytes - Phase I

The methods used for the culture of peripheral blood and splenic lymphocytes are a modification of the methods of Kligerman and coworkers (1981) and Tucker and coworkers (1986).

a. Blood Withdrawal and Lymphocyte Culture

Blood was withdrawn from CBA/H female mice 21-23 days following the start of treatment of the animals with tritiated water or X-rays by Atomic Energy of Canada Limited. Two mice from the 6 Gy tritium group were found dead on February 17, 1987 (15 days after start of treatment) and 2 others appeared unlikely to survive another day. Blood was withdrawn from these mice at this time for culturing. Blood was withdrawn after anaesthesia with chloroform in lithium heparin vacutainer tubes equipped with 20 gauge 1.5 inch vacutainer needles. The blood was mixed prior to culture by inverting the vacutainer tubes several times.

The blood was cultured in RPMI 1640 medium supplemented with 20% foetal bovine serum, 2 mM L-glutamine, 50 I.U./ml penicillin, 50 ug/ml streptomycin, 25 mM HEPES buffer, 10 I.U./ml sodium heparin, 10 uM BrdU, 55 uM 2-mercaptoethanol, 150 ug/ml ECGS and 50 ug/ml lipopolysaccharide (complete medium).

Approximately 0.2 ml aliquots of blood were added to 2.0 ml of complete RPMI medium in each well of a Linbro multi-well plate (3.5 x 1.0 mm wells). All the blood obtained from each mouse was used to establish cultures in this manner resulting in a variable number of cultures being established for each mouse. The cultures were incubated at 37 degrees C and 5% CO2-in-air for a total of 48 h. Colcemid (0.1 ug/ml) was added to each culture for a period of 4 h prior to harvest (at 44 h of total culture time).

b. Spleen Cell Preparation and Splenocyte Culture

After blood withdrawal from each mouse, the spleen was aseptically removed and placed in a sterile petri dish containing RPMI medium (unsupplemented). A single-cell suspension was prepared by aseptically mashing spleens in RPMI using the head of a syringe plunger and recovering cells by collecting the supernatant with a sterile Pasteur pipette. A uniform cell suspension was prepared by centrifugation and resuspension of the pellet in 2.0 ml of RPMI.

The culture medium used was RPMI 1640 medium supplemented with 20% foetal bovine serum, 2 mM L-glutamine, 50 I.U./ml penicillin, 50 ug/ml streptomycin, 25 mM HEPES buffer, 10 I.U./ml sodium heparin, 10 uM BrdU, 55 uM 2-mercaptoethanol, 10 ug/ml concanavalin A and 50 ug/ml lipopolysaccharide.

The cells were then counted and 10,000,000 spleen cells were added to each of 2 wells each containing 2 ml of media (2 cultures established for each mouse). Cultures were set up in Linbro multi-well plates (3.5 x 1.0 mm wells) and incubated at 37 degrees C and 5% CO2-in-air for a total culture time of 20 to 26 h. Colcemid was added to each well, 4 h prior to harvest, at a concentration of 0.1 ug/ml.

c. Harvest and Slide Preparation

The peripheral blood lymphocyte cultures were transferred into conical centrifuge tubes for harvest. The wells were then rinsed with Hank's balanced salt solution (HBSS) and the rinsates added to the individual cultures in the tubes. The cells were recovered by centrifugation at 1400 r.p.m. in an IEC HN-S Centrifuge for 6 min and the supernatant discarded. A 10 ml aliquot of 0.075 M KCl was added to the pellet in each tube and left at room temperature for 10 min. The hypotonic treatment was terminated by the addition of 5 drops of fixative (methanol : acetic acid :: 3 : 1). The cells were recovered by centrifugation and fixed with two 5 ml changes of fixative for approximately 10 min each. For overnight storage of the cells, a further 1-2 ml of fixative was added and the cells placed at 2-8 degrees C. After the final centrifugation, approximately 0.5 ml of fixative was added to the pellet to yield a suitable cell

suspension for slide preparation.

Spleen cell cultures were harvested by transferring the contents of both wells for each mouse into a conical centrifuge tube. The wells were rinsed once with HBSS and the rinsates added to the contents of the tube. The cells were recovered by centrifugation and 5 ml 0.075 M KCl added to each tube. After 5 min at room temperature the hypotonic treatment was stopped as described earlier. The cells were fixed and stored overnight in the same manner as the peripheral blood lymphocytes.

Slides were cleaned by wiping them with methanol. Cell preparations were made by breathing on a slide to create a layer of moisture and then placing 1-2 drops of cell suspension on the slide. The preparations were then allowed to air-dry for a suitable period of time.

d. Staining and Mounting

The slides were placed in a staining dish filled with a solution of Hoechst 33258 in 0.5X SSC. They were then exposed to light from a F40BL lamp at a distance of 5-6 cm for a period of 2 h. The slides were then rinsed with Sorensen's buffer, (pH 6.8, 1/15 M) placed in a staining dish filled with 2X SSC and heated at 60 degrees C for 1 h. This was followed by rinsing with Sorensen's buffer and staining with 3% Giemsa in Sorensen's buffer for 5-7 min. The slides were then

rinsed briefly with tap water and allowed to air-dry. Subsequently, they were cleared in xylene and mounted with DPX mounting media.

e. Scoring

Spleen cell slides were scored for aberrations at 1000X magnification using standard light microscopes by one of two individuals. A small number of PBL slides were also scored for aberrations. However, the quality of these latter slides was very poor and metaphases were hard to find. Therefore, scoring was aborted for the PBL slides and the results of the spleen cells used as an indicator for dose establishment for the Phase II part of the project. The slides for Phase I were not coded prior to analysis.

4. Mouse Lymphocytes - Phase II

a. Blood Withdrawal and Lymphocyte Culture

Blood was withdrawn from CBA/H female mice 29-35 days following the start of treatment of the animals with tritiated water or X-rays by Atomic Energy of Canada Limited (AECL). One mouse from the 1.5 Gy tritium group died prior to shipping from AECL. Blood was withdrawn after chloroform anaesthesia as described for Phase I.

The blood was cultured in RPMI 1640 medium supplemented with 20% foetal bovine serum, 2 mM L-glutamine, 50

I.U./ml penicillin, 50 ug/ml streptomycin, 25 mM HEPES buffer, 10 I.U./ml sodium heparin, 10 uM BrdU, 55 uM 2-mercaptoethanol, and 50 ug/ml lipopolysaccharide (complete medium). Some of the later cultures (those established on May 9, 10 and 11 - see Appendix II, Phase II Schedule) also received 10 ug/ml concanavalin A. This was added to cultures at this stage to try to stimulate mitosis further in the lymphocytes, since the mitotic index, on cursory evaluation, appeared to be somewhat low in the cultures from previous days.

Lymphocyte cultures were established as described for Phase I, except that the cultures were incubated for a total of 49 h and colcemid was added after 45 h, 4 h prior to harvest.

b. Spleen Cell Preparation and Splenocyte Culture

A single-cell spleen suspension was prepared as described for Phase I. The culture medium used was also the same. The only deviation from the Phase I procedure was that the cultures were incubated for a total of 26 h and colcemid was added at 22 h, 4 h prior to harvest.

c. Harvest and Slide Preparation

The lymphocytes from the cell cultures were recovered as described for Phase I. Thereafter, a 5 ml aliquot of 0.075 M KCl was added to each tube and left at room

temperature for 10 min. This treatment was terminated with a few drops of fixative as before. The cells were recovered by centrifugation and resuspended in 5 ml of fresh fixative. After 10 min at room temperature, the cells were recovered by centrifugation. The supernatant was discarded and 4 ml of fresh fixative was put into one tube from each culture group. The cells were resuspended and this suspension was used to pool the cells from all the cultures of one mouse into one tube. The cells were then left at room temperature in fixative for a minimum of 10 min prior to their recovery by centrifugation. A small volume of fresh fixative was added to each tube in order to yield a suitable cell suspension for slide preparation.

Spleen cells were harvested as described for Phase I. The hypotonic treatment was performed using 4 ml of 0.075 M KCl for 5 min at room temperature. Hypotonic treatment was stopped as described earlier and the cells fixed twice in 5 ml, and then 4 ml, of fresh fixative for 10 min each time. A suitable cell suspension was then prepared for making slides.

Slides were cleaned and cells spread on the slides as described in Phase I.

d. Staining and Mounting

The slides were stained with Giemsa and the preparations mounted using DPX mounting media as

described for Phase I.

e. Randomisation and Scoring

Lymphocyte slides were evaluated for the quality of the preparations and for the presence of a sufficient number of metaphase figures. From the better quality preparations, the requisite number of slides were chosen from each dose group for analysis. The slides were then randomised and coded by an individual who was not involved in scoring. The slides were then scored by one or the other of two individuals at 1000X magnification using standard light microscopes. The spleen cell slides were neither randomised nor scored.

5. Mouse Spermatocytes

a. Removal of Testes and Collection of Spermatocytes

The mice were sacrificed by cervical dislocation starting at 77 days and ending at 86 days after initiation of treatment with tritiated water. One mouse was sacrificed on day 70 from the 4.5 Gy X-ray treatment group as a trial to determine appropriateness of time for harvest. For another trial one mouse was sacrificed on day 71 from each of the 4.5 Gy X-ray and tritium treatment groups. The reason for waiting approximately 70 days is that radiation-induced toxicity leads to a lowered rate of cell division. It is necessary to allow the animals to recover from this

toxicity prior to collection of spermatocytes for analysis. At 70 days, the meiotic cells are cells that were primary spermatocytes at the time of irradiation. They thus represent the developmental stage that is present for most of the adult life and consequently dominates the risk.

The testes were placed in a petri dish containing 2.2% tri-sodium citrate and rinsed thoroughly to remove any fat. They were then transfered into another dish containing a small volume of fresh 2.2% tri-sodium citrate and the tunica removed. The tubules were cut, teased, and triturated to release the spermatocytes and the total volume made up to 10 ml with 2.2% tri-sodium citrate. The tubules were allowed to settle for a few minutes and the supernatant then transferred into a fresh tube. An additional volume of 2.2% tri-sodium citrate was added to rinse the tubules and the supernatant retained as before. This method is a modification of the method of Evans and coworkers (1964).

b. Harvest and Slide Preparation

The cells were recuperated by centrifugation at 1400 r.p.m. in a bench top centrifuge for 6 min. A 10 ml volume of 1% tri-sodium citrate was used to resuspend the pellet with the help of a Pasteur pipette. Hypotonic treatment was continued at room temperature

for a period of 10 min. The cells were then recovered by centrifugation as before and resuspended in 10 ml of fixative (as previously defined) by tapping the tube. The suspension was then mixed well using a Pasteur pipette. After 10 min at room temperature, the fixative was replaced with a 5 ml volume of fresh fixative following centrifugation. After a further 10 min, the cells were recentrifuged. A volume of fresh fixative was added to yield an appropriate cell suspension for slide preparation. A layer of moisture was created on each slide by breathing on it. A few (2-3) drops of cell suspension were placed on the slide and spread by rotational tilting. The excess was drained on absorbent paper prior to air-drying.

c. Staining and Mounting

The slides were stained with 5% Giemsa in Sorensen's buffer for 6 min and then rinsed briefly with tap water. Subsequently, they were allowed to air-dry, cleared in xylene and mounted with DPX mounting media.

d. Randomisation and Scoring

The slides were evaluated for the quality of the preparations and for the presence of a sufficient number of meiotic cells. The requisite number of slides were chosen from the better quality slides in each group, and randomised by an individual not involved in the scoring. The slides were then scored

by one or the other of two individuals at 1000X magnification using standard light microscopes. In order to minimize any variation due to scoring by two individuals, the scoring of the two individuals was compared and standardized on trial slides prior to scoring the aberrations in both the lymphocytes and the spermatocytes.

First division metaphases only were used for analysis of chromosome and chromatid aberrations. Differential staining of chromosomes by the fluorescence plus Giemsa technique (Perry and Wolff, 1974) enabled the differentiation of first division metaphases from subsequent cell divisions.

6. Statistical Analysis

Two statistical analyses were performed. The initial analysis, which forms the basis for the body of the report, is described below. A second analysis by a well known statistician with considerable expertise in the field was commissioned and is included as Appendix XII. The conclusions drawn as to the values of the Relative Biological Effectiveness and the associated uncertainties are based on the second analysis by Dr. Margolin and his associate. In their report, they noted that there were three minor discrepancies in the data provided to them and that they assumed that the tabular data were correct. We

have checked these points and found that they were correct in this assumption. Hence their analysis was performed with the correct data. The following section describes the method used for the primary analysis upon which the figures in the main body of the report are based.

The results obtained were analysed by first converting all dose group totals into a frequency per 100 cells. This frequency is expressed as a mean $\pm$ S.E.M. of all the data points within each dose group. Results are shown in tables as the total number of each aberration-type analysed at each data point as well as the frequency for each dose group, where

$$\text{S.E.M.} = \frac{\text{S.D.}}{\sqrt{N}}$$

and N = number of animals (slides) per dose group.

The spectrum of aberrations noted in peripheral blood lymphocytes and primary spermatocytes are shown in the tables. However, only the following categories were analysed for determination of a radiation dose-response effect: dicentrics and centric rings, terminal deletions, interstitial deletions, and acentric rings from peripheral blood lymphocytes, and translocations for primary spermatocytes. Only chromosome-type aberrations were analysed for a dose-response relationship since these are the most sensitive indicator of radiation-induced damage. Other aberrations were noted but not used in the determination of relative biological effectiveness.

A weighted linear regression analysis was carried out on the data obtained in the Phase I range-finding study using the total number of each aberration group as well as the total number of cells for each dose group. The slopes were calculated for both types of lymphocytes and are noted in the appropriate tables. Weighted linear regression analyses were carried out on the data obtained in the Phase II studies using individual doses, individual number of cells, as well as the individual number of aberrations for each mouse per slide analyzed, such that each mouse represents a separate data point. The weighted linear regression was carried out based on the following equation:

$$y = a + bx$$

where b is the slope, x is the dose, a (the intercept) is the spontaneous aberration frequency and y is the aberration frequency at dose x. The equation for the weighted linear regression is:

$$\bar{Y} = a + b\bar{X}$$

where

$$\bar{Y} = \frac{\Sigma y_i}{N}$$

and

$$\bar{X} = \frac{\Sigma n_i X_i}{N}$$

Also $$a = \bar{Y} - b\bar{X}$$

and $$b = \frac{\Sigma n_i x_i y_i}{\Sigma n_i x_i^2}$$

$$= \frac{\Sigma X_i Y_i - (\Sigma n_i X_i)(\Sigma Y_i) / N}{\Sigma n_i X_i^2 - (\Sigma n_i X_i)^2 / N}$$

where

$\bar{Y}$ = weighted mean frequency of aberrations per cell

a = intercept or spontaneous aberration frequency per cell

b = slope

$\bar{X}$ = weighted mean dose

y_i = individual number of aberrations per animal (slide)

N = total number of cells scored for all the animals (slides)

n_i = number of cells scored per animal (slide)

X_i = individual animal dose

x_i = deviation from weighted mean dose

$\bar{y}_i$ = deviation from weighted mean frequency of aberrations

Y_i = number of aberrations per group of animals having received dose X_i

Reference: Snedecor and Cochran, 1980

6. Data Retention

All data, including the reports, raw data sheets and slides from this study, will be retained for at least 5 years in the archives of Bio-Mutatech Inc., to be made available for inspection, upon request, by authorised personnel of the sponsors.

RESULTS

A. Effect of X- and Tritium Beta-irradiation on the Induction of Chromosomal Aberrations in Spleen and Peripheral Blood Lymphocytes in the Phase I Range-finding Study

The method development study showed that spleen cells were very easy to culture and yielded a very high mitotic index in comparison to peripheral blood lymphocytes. Therefore, both splenic as well as peripheral blood lymphocytes were cultured for Phase I and II of the Project. The splenic lymphocytes served as a suitable alternate in case the cultures of the peripheral blood lymphocytes did not yield a sufficiently high mitotic index for the required analysis.

Upon evaluation of the splenic and peripheral blood lymphocyte slides, the spleen cells were determined to be of superior quality in terms of the mitotic index and of metaphase preparations. The difference in the mitotic indices of the spleen versus the peripheral blood lymphocytes is exemplified in Figures 1 and 2. These figures clearly show the dramatic difference in the availability of mitoses for analysis. Thus, it was decided to use the spleen cell slides for evaluation of the chromosomal damage induced by tritium and X-irradiation in the Phase I range-finding part of the study. In addition, the better quality of the splenic lymphocyte preparations dramatically increased the efficiency of scoring. When the scoring of the spleen cells was completed, a small number of peripheral blood lymphocyte slides were scored from each dose group. This was performed in order to determine whether splenic lymphocytes and peripheral blood lymphocytes yielded comparable results and

could, therefore, be used interchangeably.

The results for chromosomal aberrations observed in splenic lymphocytes are shown in Tables 1 - 5. The full spectrum of aberrations (chromosome and chromatid types) noted for individual animals are shown in separate tables for each dose group as follows:

Table 1 : controls,
Table 2 : 2 Gy tritium,
Table 3 : 6 Gy tritium,
Table 4 : 2 Gy X-rays, and
Table 5 : 6 Gy X-rays.

Table 6 summarises the specific aberrations normally used in the calculation of relative biological effectiveness. These are grouped together for evaluation as follows:

Dicentrics and centric rings,
Terminal deletions, and
Interstitial deletions and acentric rings.

The results from this phase of the study are discussed on the assumption that the doses administered were in fact 2.0 and 6.0 Gy for the respective treatment groups. It should be noted, however, that no definite conclusions regarding relative biological effectiveness could be drawn from these results since only the nominal doses were available to us. In addition, the purpose of this phase of the study was only to determine suitable doses to be used in Phase II.

Figures 3 - 5 show the graphical representation of the data obtained for the above-defined groups of aberrations for both tritium and X-ray exposures. The weighted linear

regression lines for these aberration frequencies (per 100 cells) are plotted along with the mean ± S.E.M. for each dose point.

A comparison was made between the slopes of the linear regression curves for the induction of spleen cell aberrations by tritium and X-rays. The results show that the slope for tritium is greater than the slope for X-rays.

In addition to these types of aberrations, increases were also observed in the induction of chromosome translocations (tritium: 39/500; X-rays: 8/400), chromosome gaps (tritium: 5/500; X-rays: 0/400) and pericentric inversions (tritium: 6/500; X-rays: 1/400) at the 6.0 Gy dose. Chromatid deletions were also increased at the 2.0 Gy dose (tritium: 12/500; X-rays: 3/500) and at the 6.0 Gy dose (tritium: 79/500; X-rays: 11/400). This may be the result of some cycling of splenic lymphocytes *in vivo*, residual radiation during culture, or persistent radiation damage.

To evaluate the range of aberrations induced by tritium and X-rays in the lymphocytes of peripheral blood, 200 cells were scored from each dose group (except for the 6.0 Gy tritium dose group where only 192 cells were observed from the 3 animals evaluated). This was performed in order to determine whether the spleen and peripheral blood lymphocytes displayed comparable aberration frequencies and could therefore substitute for each other. The full spectrum of aberrations in the peripheral blood lymphocytes noted for

the individual animals for each dose group are shown in the following tables:

Table 7 : controls,
Table 8 : 2.0 Gy tritium,
Table 9 : 6.0 Gy tritium,
Table 10 : 2.0 Gy X-rays, and
Table 11 : 6.0 Gy X-rays.

Table 12 summarises the specific aberration types traditionally used to calculate relative biological effectiveness. These groups are as defined earlier for Table 6. The results are shown as the mean ± S.E.M. of the frequencies for these aberration types and are graphically represented in Figures 6 - 8 for each dose group. The weighted linear regression lines for the aberration frequencies noted in Phase II for peripheral blood lymphocytes are plotted on these graphs to demonstrate the level of concordance between the data obtained for the two phases.

The weighted linear regression curves from the data of Phase I show that tritium appears to induce a greater number of aberrations compared to X-rays of the same putative dose.

	Tritium/X-rays
Dicentrics and centric rings	2.94
Terminal deletions	1.17
Interstitial deletions and acentric rings	3.55

In addition a noticeable increase was seen in the number of chromosomal translocations at the 6.0 Gy tritium dose (tritium: 7/192; X-rays: 1/200). The number of chromatid deletions also increased at the 2.0 Gy dose levels (tritium: 8/192; X-rays: 5/200). At the 6.0 Gy dose the number of chromatid deletions induced by both tritium and X-rays was found to be 2/200.

A comparison of the weighted linear regression results obtained using the spleen lymphocytes vs the peripheral blood lymphocytes show that the ratios of the slopes of tritium/X-rays (putative relative biological effectiveness) are similar for dicentrics and centric rings and for interstitial deletions and acentric rings. The terminal deletions show a 2-fold greater slope with spleen cells in comparison to peripheral blood lymphocytes.

A comparison of the aberration frequencies (mean $\pm$ S.E.M. per 100 cells) obtained with spleen cells vs peripheral blood lymphocytes show an approximately 4-fold increase in the frequency of dicentrics and centric rings and of interstitial deletions and acentric rings. The frequency of terminal deletions is increased approximately 3-fold in spleen cells only at the 6.0 Gy tritium dose. All the other doses (2.0 Gy tritium and both the X-ray doses) show very similar frequencies.

No quantitative data were collected for determination of the mitotic index for each dose. However, we observed that the

mitotic index decreased dramatically at the 6.0 Gy dose. This decrease was greater with tritium treatment than with X-ray treatment at this dose. Based upon these observations and the aberration frequency data obtained, we considered that 6.0 Gy would be too toxic (inhibitory for cell division) for use in Phase II as the highest dose. Therefore, the doses suggested for use in Phase II were 1.5, 3.0, and 4.5 Gy since these would be expected to allow cell division at rates commensurate with those required to yield a sufficient number of cells for metaphase analysis.

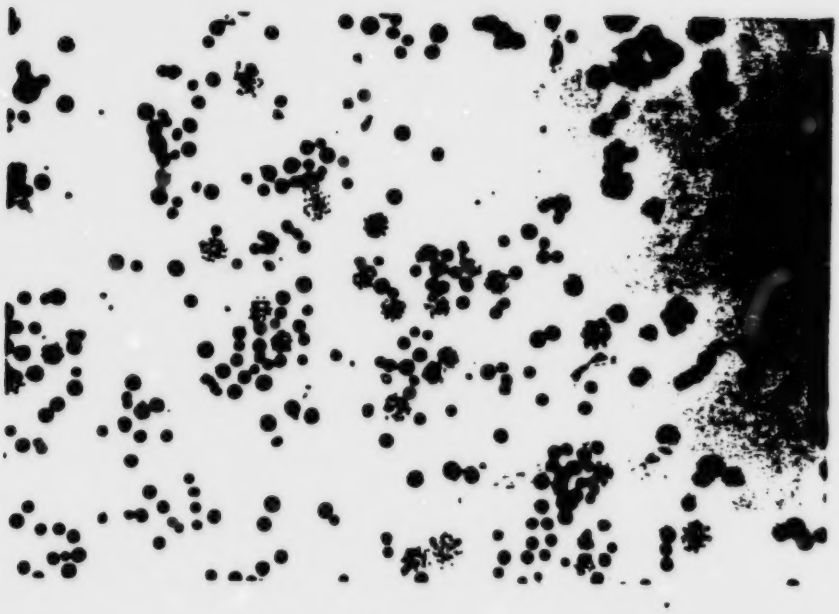

Figure 1. Typical low magnification field of a mitotic preparation of CBA/H mouse splenic lymphocytes (160 X)

Figure 2. Typical low magnification field of a mitotic preparation of CBA/H mouse peripheral blood lymphocytes (160 X)

TABLE 1 Spontaneous Chromosomal Aberrations in the Splenic Lymphocytes of Unirradiated Control CBA/H Mice - Phase I

Animal Code	Total Number Of Cells Scored	Number Of Normal Cells	Aberrations										
			Chromosome					Chromatid					
			Dic	Ring	Del	ID	Other	Inter	Iso	Del	ID	Other
1	100	99			1							
3	100	97						1 SI		2		1 gap
4	100	96			2				1	1		
5	100	98			1					1		
11	100	98			1							1 gap
12	100	98			2							
13	100	99								1		
14	100	95			2		1 PCD			2		
15	100	98								1		1 gap
Totals	900	878			9		1 PCD	1 SI	1	8		3 gap

TABLE 2 Chromosomal Aberrations in the Splenic Lymphocytes of CBA/H Female Mice Treated with a Nominal 2.0 Gy Dose of Tritiated Water - Phase I

Animal Code	Total Number Of Cells Scored	Number Of Normal Cells	Aberrations									
			Chromosome					Chromatid				
			Dic	Ring	Del	ID	Other	Inter	Iso	Del	ID	Other
16	100	69	29	1 ac	9	3	2 trans		1	1		
17	100	71	18		12		1 trans			4	2	
21	100	86	4		9		1 trans		1	4		
22	100	82	9	1 ac	16		2 gap		1	2	1	
23	100	84	4		10		3 trans		1	1		1 gap
Totals	500	392	64	2 ac	56	3	7 trans 2 gap		4	12	3	1 gap

TABLE 3 Chromosomal Aberrations in the Splenic Lymphocytes of CBA/H Female Mice Treated with a Nominal 6.0 Gy Dose of Tritiated Water - Phase I

Animal Code	Total Number Of Cells Scored	Number Of Normal Cells	Aberrations								
			Chromosome					Chromatid			
			Dic	Ring	Del	ID	Other	Inter	Iso	Del	ID	Other
26	100	5	100	2 ac	84	43	11 trans 2 gap 1 PI 1 PCD		3	18		
27	100	7	137	1 c 3 ac	88	3	18 trans 2 gap 4 PI 1 PCD		1	37		3 gap
28	100	65	33	3 ac	27	9	1 trans		2	11		
30	100	68	24	1 c 3 ac	15	3	4 trans			9		
31	100	80	18		8	4	5 trans 1 PI 1 gap			4		
Totals	500	225	312	11 ac 2 c	222	62	39trans 5 gap 6 PI 2 PCD		6	79		3 gap

TABLE 4 Chromosomal Aberrations in the Splenic Lymphocytes of CBA/H Female Mice Treated with a Nominal 2.0 Gy Dose of X-rays - Phase I

Animal Code	Total Number Of Cells Scored	Number Of Normal Cells	Aberrations									
			Chromosome					Chromatid				
			Dic	Ring	Del	ID	Other	Inter	Iso	Del	ID	Other
32	100	82	6		5	3	1 trans			1		1 gap
33	100	80	7	1 ac	8	1	1 trans 2 PI			2		
34	100	90	5		3			2				
37	100	92	3	1 ac	5		1 gap					1 gap
39	100	92			6	2		2				
Totals	500	436	21	2 ac	27	6	2 trans 1 gap 2 PI	4		3		2 gap

TABLE 5 Chromosomal Aberrations in the Splenic Lymphocytes of CBA/H Female Mice Treated with a Nominal 6.0 Gy Dose of X-rays - Phase I

Animal Code	Total Number Of Cells Scored	Number Of Normal Cells	Aberrations								
			Chromosome					Chromatid			
			Dic	Ring	Del	ID	Other	Inter	Iso	Del	ID	Other
41	100	60	23	3 ac	26	6		1	2	3		
42	100	60	26	1 c 1 ac	18	2	3 trans	2	4			
46	100	70	17		18	5	2 trans	1	2			
47	100	75	8		11	1	3 trans 1 PI	1	3			
Totals	400	265	74	4 ac 1 c	73	14	8 trans 1 PI	5	11	3		

TABLE 6 Summary Table of Dicentrics and Acentrics in CBA/H Mouse Splenic Lymphocytes - Phase I

Dose Group	Number of Cells Scored	Total # of Chromosome-Type Aberrations*		
		Dicentrics + Centric Rings	Terminal Deletions	Interstitial Deletions and Acentric Rings
Control	900	0	9 (1.00 ± 0.29)	0
Tritium				
2.0 Gy	500	64 (12.8 ± 4.79)	56 (11.2 ± 1.32)	5 (1.00 ± 0.77)
6.0 Gy	500	314 (62.8 ± 23.8)	222 (44.4 ± 17.3)	73 (14.6 ± 7.72)
X-rays				
2.0 Gy	500	21 (4.20 ± 1.24)	27 (5.40 ± 0.81)	8 (1.60 ± 0.51)
6.0 Gy	400	75 (18.8 ± 4.13)	73 (18.3 ± 3.07)	18 (4.50 ± 1.71)
Slopes from weighted linear regressions				
Tritium		0.1006	0.0722	0.0224
X-rays		0.03	0.03	0.00756

* The numbers within parentheses represent the mean ± S.E.M. of the aberrations per 100 cells (frequency)

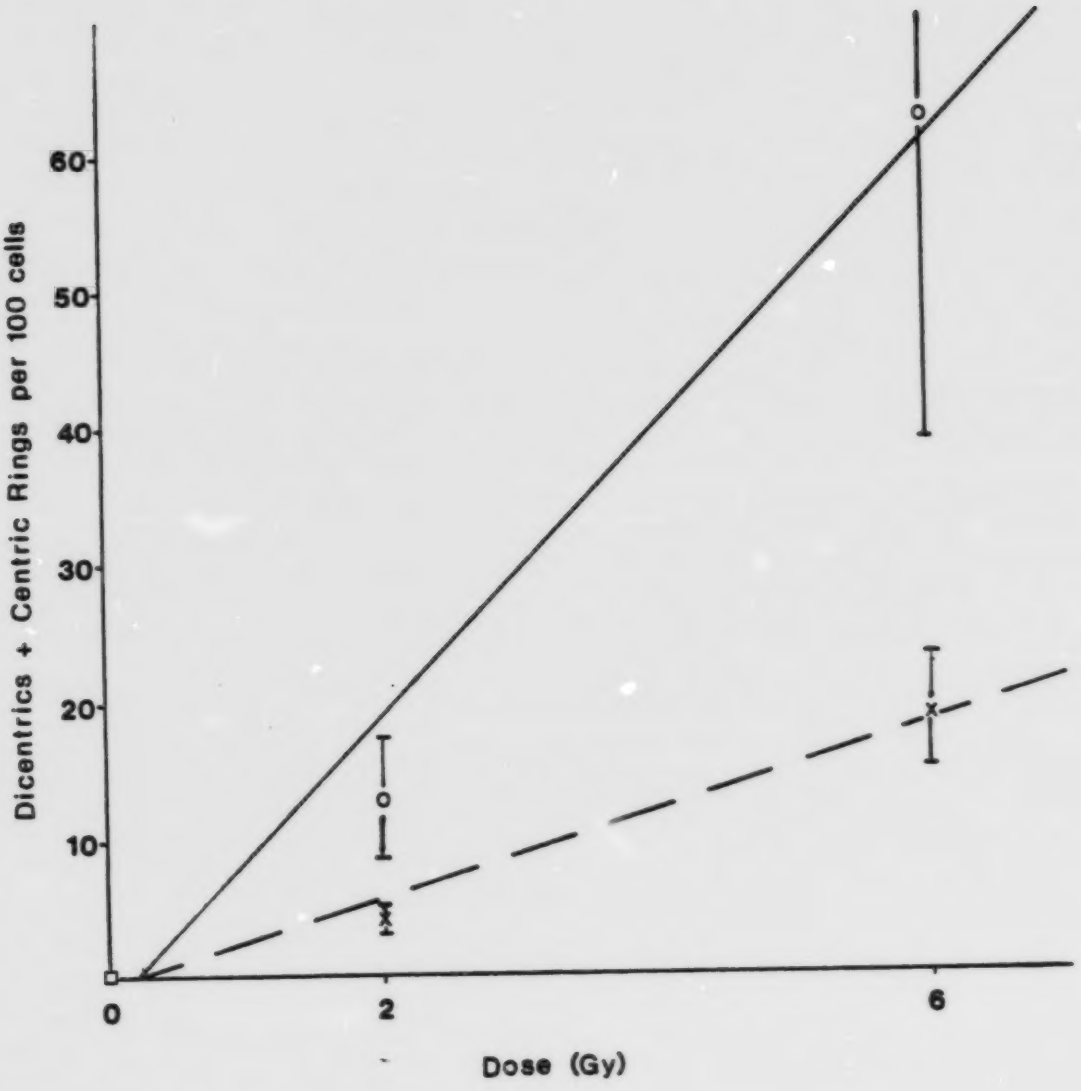

Figure 3. Frequency (mean ± S.E.M.) of dicentrics and centric rings in CBA/H mouse splenic lymphocytes treated with tritium (HTO) and X-irradiation with the weighted linear regression lines - Phase I range-finding study

Legend: Spontaneous frequency: □
Tritium: o, solid line; X-rays: x, dashed line
Vertical error bars indicate the S.E.M. of the frequency.

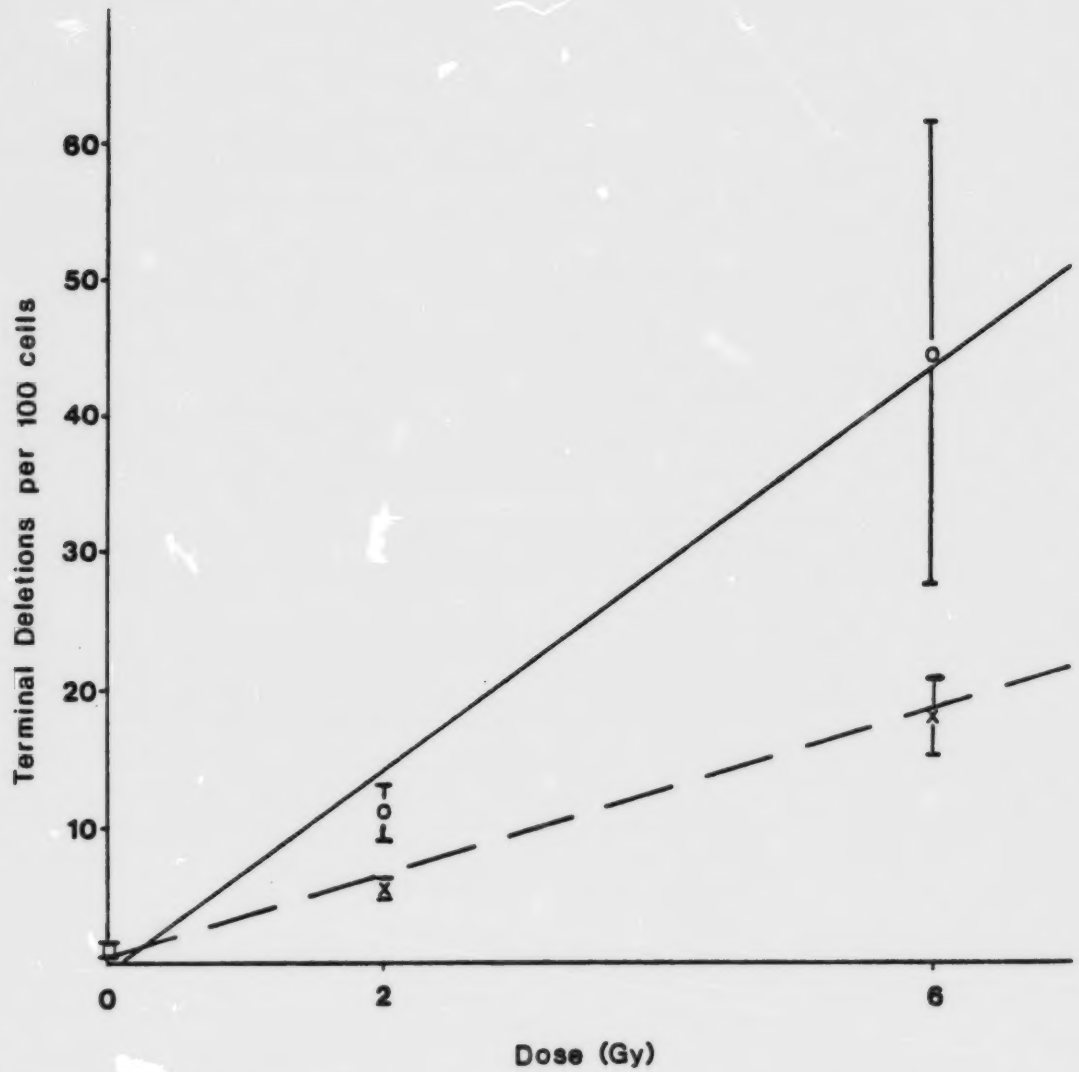

Figure 4. Frequency (mean ± S.E.M.) of terminal deletions in CBA/H mouse splenic lymphocytes treated with tritium (HTO) and X-irradiation with the weighted linear regression lines - Phase I range-finding study

Legend: Spontaneous frequency: □
Tritium: o, solid line; X-rays: x, dashed line
Vertical error bars indicate the S.E.M. of the frequency.

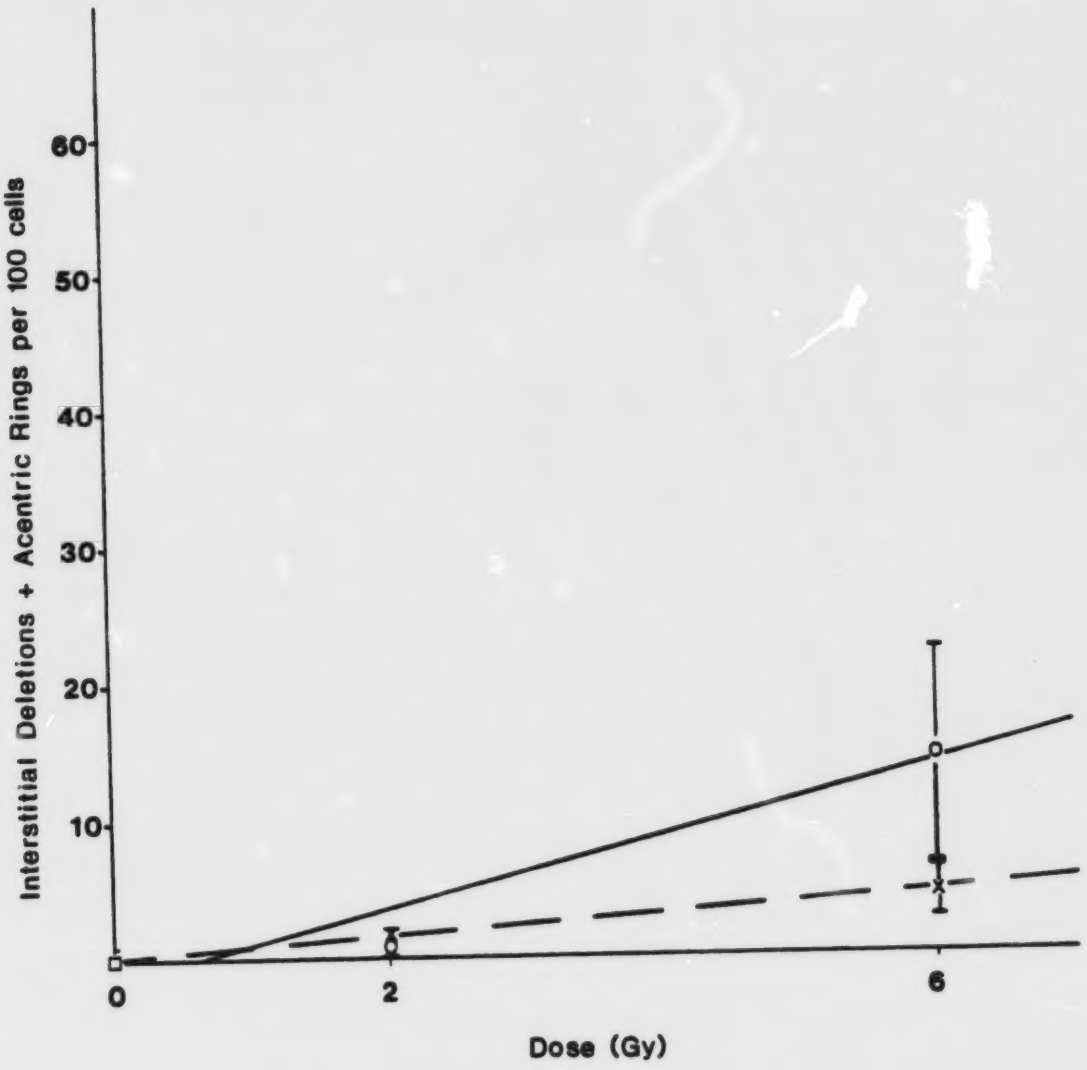

Figure 5. Frequency (mean ± S.E.M.) of interstitial deletions and acentric rings in CBA/H mouse splenic lymphocytes treated with tritium (HTO) and X-irradiation with the weighted linear regression lines - Phase I range-finding study

Legend: Spontaneous frequency: □
Tritium: o, solid line; X-rays: x, dashed line
Vertical error bars indicate the S.E.M. of the frequency.

TABLE 7 Spontaneous Chromosomal Aberrations in the Peripheral Blood Lymphocytes of Unirradiated Control CBA/H Female Mice - Phase I

Animal Code	Total Number Of Cells Scored	Number Of Normal Cells	Aberrations									
			Chromosome					Chromatid				
			Dic	Ring	Del	ID	Other	Inter	Iso	Del	ID	Other
1	100	98			1	1						
3	100	99					1 PCD					
Totals	200	197			1	1	1 PCD					

TABLE 8 Chromosomal Aberrations in the Peripheral Blood Lymphocytes of CBA/H Female Mice Treated with a Nominal 2.0 Gy Dose of Tritiated Water - Phase I

Animal Code	Total Number Of Cells Scored	Number Of Normal Cells	Aberrations									
			Chromosome					Chromatid				
			Dic	Ring	Del	ID	Other	Inter	Iso	Del	ID	Other
16	100	87	3		11		3 PCD			2		
17	100	91	1		7		1 trans					
Totals	200	178	4		18		3 PCD 1 trans			2		

TABLE 9 Chromosomal Aberrations in the Peripheral Blood Lymphocytes of CBA/H Female Mice Treated with a Nominal 6.0 Gy Dose of Tritiated Water - Phase I

Animal Code	Total Number Of Cells Scored	Number Of Normal Cells	Aberrations									
			Chromosome					Chromatid				
			Dic	Ring	Del	ID	Other	Inter	Iso	Del	ID	Other
28	100	73	13		20	6	4 trans		5			
29	46	37	5		2	1	1 trans		1			
31	46	33	10		8	1	2 trans 1 PCD		2	1		
Totals	192	143	28		30	8	7 trans 1 PCD		8	1		

TABLE 10 Chromosomal Aberrations in the Peripheral Blood Lymphocytes of CBA/H Female Mice Treated with a Nominal 2.0 Gy Dose of X-rays - Phase I

Animal Code	Total Number Of Cells Scored	Number Of Normal Cells	Aberrations									
			Chromosome					Chromatid				
			Dic	Ring	Del	ID	Other	Inter	Iso	Del	ID	Other
32	100	90	1	1 ac	6		1 trans			1		
33	100	89	3		6		1 PCD			1		
Totals	200	179	4	1 ac	12		1 trans 1 PCD			2		

TABLE 11 Chromosomal Aberrations in the Peripheral Blood Lymphocytes of CBA/H Female Mice Treated with a Nominal 6.0 Gy Dose of X-rays - Phase I

Animal Code	Total Number Of Cells Scored	Number Of Normal Cells	Aberrations								
			Chromosome					Chromatid			
			Dic	Ring	Del	ID	Other	Inter	Iso	Del	ID	Other
41	100	85	4		12	1				1		
46	50	38	3		8	1	1 trans		2	3		
47	50	40	2		8					1		
Totals	200	163	9		28	2	1 trans		2	5		

TABLE 12 Summary Table of Dicentrics and Acentrics in CBA/H Mouse Peripheral Blood Lymphocytes - Phase I

Dose Group	Number of Cells Scored	Total # of Chromosome-type Aberrations*		
		Dicentrics + Centric Rings	Terminal Deletions	Interstitial Deletions and Acentric Rings
Control	200	0	1 (0.50 ± 0.50)	1 (0.50 ± 0.50)
Tritium 2.0 Gy	200	4 (2.00 ± 1.00)	18 (9.00 ± 2.00)	0
6.0 Gy	192**	28 (15.2 ± 3.33)	30 (13.9 ± 4.84)	8 (3.45 ± 1.28)
X-rays 6.0 Gy	200	4 (2.00 ± 1.00)	12 (6.00 ± 0.00)	1 (0.50 ± 0.50)
2.0 Gy	200	9 (4.50 ± 0.50)	28 (14.0 ± 2.00)	2 (1.00 ± 0.00)
Slopes from weighted linear regressions				
Tritium		0.02282	0.02801	0.00622
X-rays		0.00775	0.024	0.00175

* The numbers within parentheses represent the mean ± S.E.M. of the aberrations per 100 cells (frequency).

** Only 192 cells were scored instead of 200 because no more cells could be found on any slides from all the mice treated at this dose.

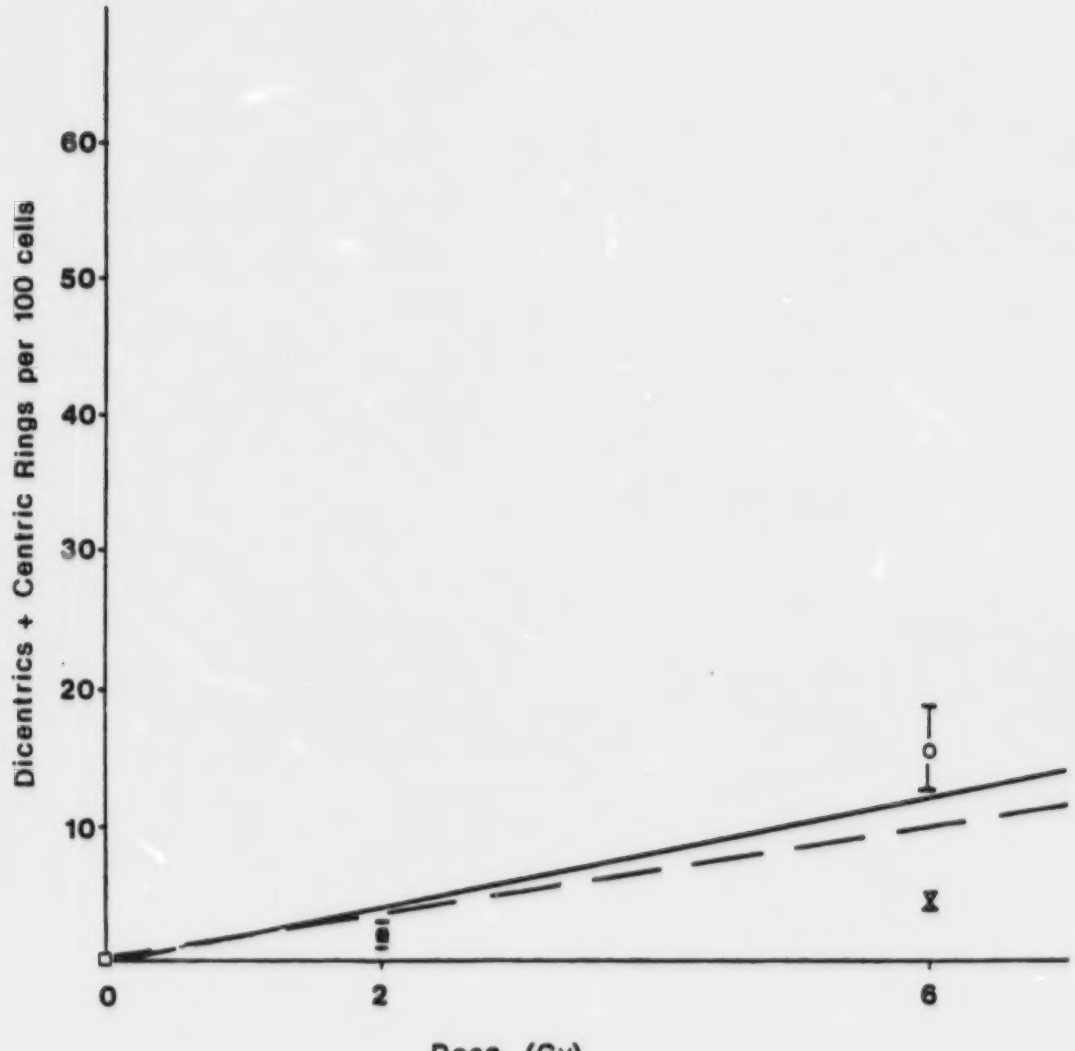

Figure 6. Frequency (mean ± S.E.M.) of dicentrics and centric rings in CBA/H mouse peripheral blood lymphocytes treated with tritium (HTO) and X-irradiation - Phase I range-finding study

The weighted linear regression lines plotted on this graph are from the corresponding Phase II peripheral blood lymphocyte portion of the study.

Legend: Spontaneous frequency: □
Tritium: o, solid line; X-rays: x, dashed line
Vertical error bars indicate the S.E.M. of the frequency.

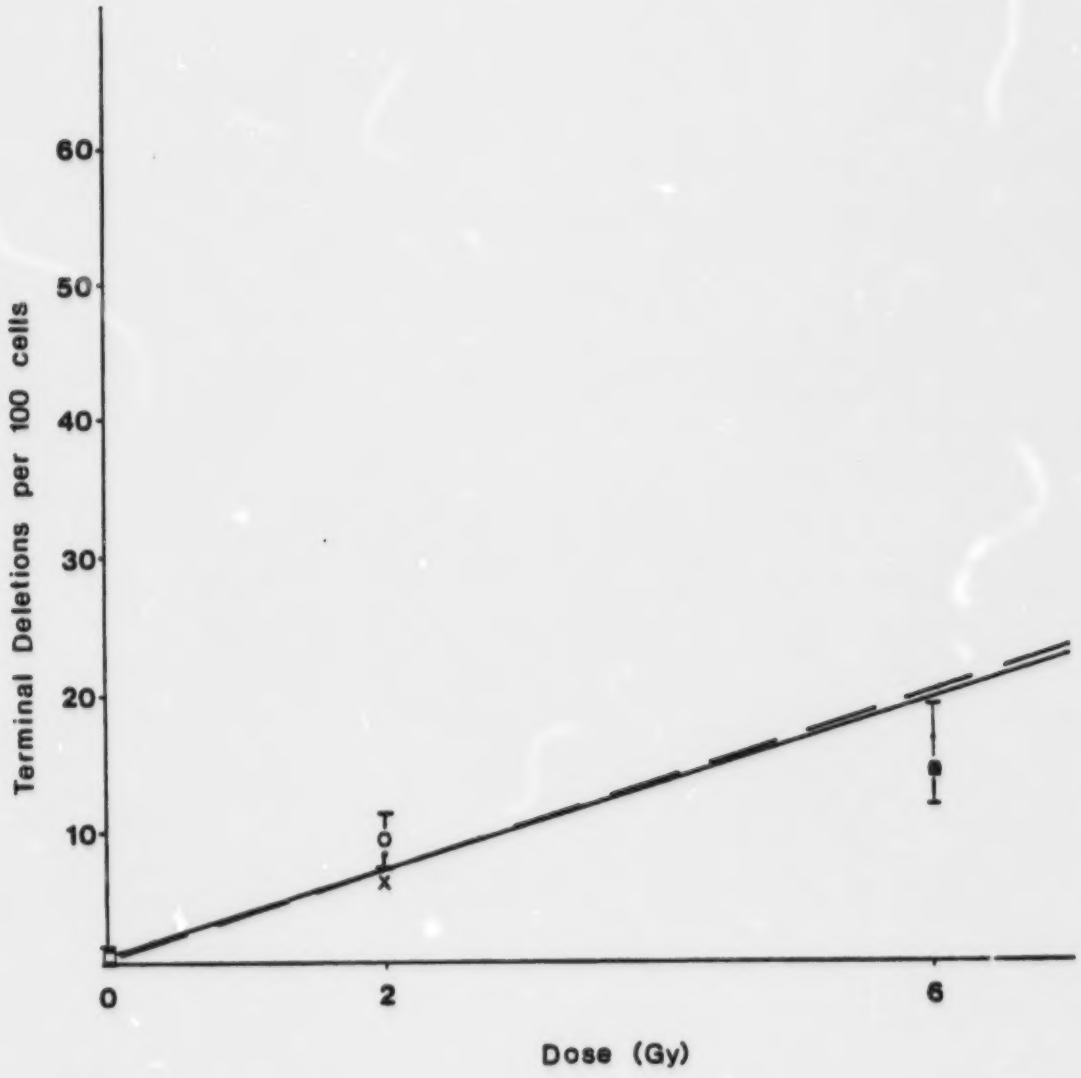

Figure 7. Frequency (mean ± S.E.M.) of terminal deletions in CBA/H mouse peripheral blood lymphocytes treated with tritium (HTO) and X-irradiation - Phase I range-finding study

The weighted linear regression lines plotted on this graph are from the corresponding Phase II peripheral blood lymphocyte portion of the study.

Legend: Spontaneous frequency: □
Tritium: o, solid line; X-rays: x, dashed line
Vertical error bars indicate the S.E.M. of the frequency.

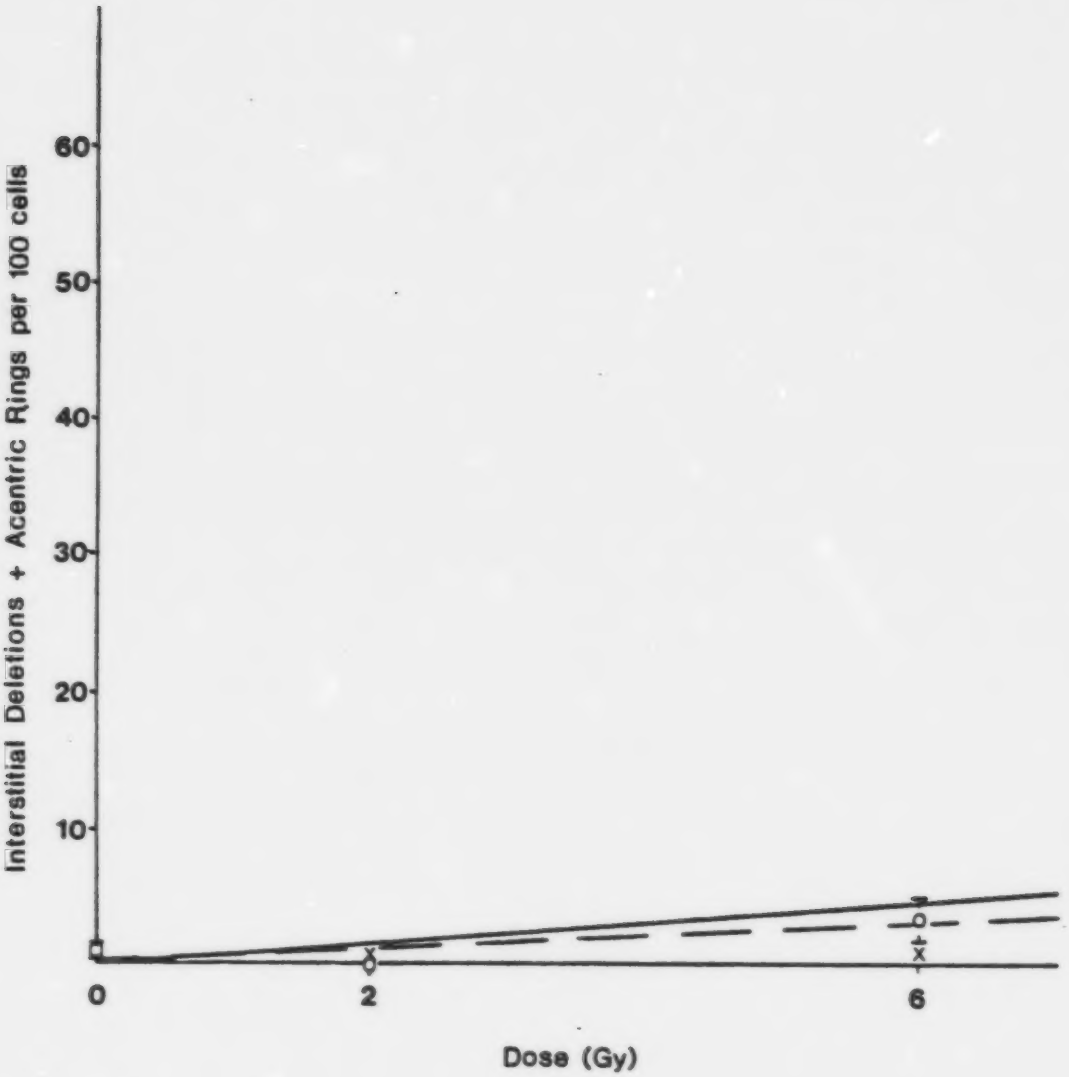

Figure 8. Frequency (mean ± S.E.M.) of interstitial deletions and acentric rings in CBA/H mouse peripheral blood lymphocytes treated with tritium (HTO) and X-irradiation - Phase I range-finding study

The weighted linear regression lines plotted on this graph are from the corresponding Phase II peripheral blood lymphocyte portion of the study

Legend: Spontaneous frequency: □
Tritium: o, solid line; X-rays: x, dashed line
Vertical error bars indicate the S.E.M. of the frequency.

B. Effect of Tritium and X-rays on the Induction of Chromosomal Aberrations in the Peripheral Blood Lymphocytes of the CBA/H Mouse for Phase II

For the Phase II part of the study, both peripheral blood and spleen lymphocytes were cultured from each animal. Only the peripheral blood lymphocytes have been evaluated thus far for radiation-induced chromosomal damage. The spleen cells were cultured as an alternate system for analysis in case the peripheral blood lymphocytes should yield too few mitotic cells for analysis. The slides were not used but have been stored in case they should be required.

As a result of culturing spleen lymphocytes, data were accumulated regarding the total spleen cell number per animal from each dose group. This data now serves as a measure of radiation-induced reduction in spleen size. The extent of this toxicity in the different dose groups is shown as follows:

	Theoretical Dose	Actual Dose	% Reduction in Mean Spleen Cell Number
Tritium	1.5 Gy	1.52 Gy	37.5
	3.0 Gy	3.87 Gy	60.7
	4.5 Gy	5.36 Gy	59.4
X-rays	1.5 Gy	1.33 Gy	34.5
	3.0 Gy	2.82 Gy	42.2
	4.5 Gy	3.36 Gy	52.8

The full spectrum of chromosomal aberrations are listed in Tables 13 - 19 for all the individual animals in each of the

following dose groups:

Table 13	:	controls,
Table 14	:	1.5 Gy tritium,
Table 15	:	3.0 Gy tritium,
Table 16	:	4.5 Gy tritium,
Table 17	:	1.5 Gy X-rays,
Table 18	:	3.0 Gy X-rays, and
Table 19	:	4.5 Gy X-rays.

For these animals, individual dosimetry data was provided by Atomic Energy of Canada Limited and is shown in Appendix XI.

The animals used for the Phase II lymphocyte study ranged in weight at the different dose levels as follows:

Nominal Dose (Gy)	Weight Range (g)	Mean ± S.E.M.
1.5	17.9 - 27.8	24.0 ± 0.47
3.0	18.8 - 23.1	21.9 ± 0.37
4.5	21.3 - 27.3	24.8 ± 0.68

The tritium dose administered as tritiated water to the animals of each of the three dose groups were as follows:

Nominal Dose (Gy)	Injected (uCi/mouse)
1.5	4077.0
3.0	8153.0
4.5	12230.0

The tissue dose to each animal of the tritium and X-ray dose groups ranged as follows:

Nominal Dose (Gy)	Dose Range (Gy)	Mean ± S.E.M.
Tritium		
1.5	1.31 - 1.80	1.52 ± 0.04
3.0	3.46 - 4.74	3.87 ± 0.10
4.5	3.49 - 6.74	5.36 ± 0.30
X-rays		
1.5	N/A	1.33 ± 0.01
3.0	N/A	2.82 ± 0.03
4.5	N/A	3.36 ± 0.18

The results of Phase II, shown in Tables 13 - 19, once again confirmed the general results observed in Phase I. Tritium-treated animals showed considerably greater numbers of aberrations per cell scored compared to X-ray-treated animals. These data are tabulated for the traditional aberration types used in the calculation of relative biological effectiveness and are shown in summary Table 20. The aberrations are listed as the total number of aberrations per dose group and as the mean ± S.E.M. per 100 cells. A weighted linear regression analysis was carried out using the individual animal dosimetric data for both the tritium and X-ray groups.

Figures 9 - 11 show a graphical representation of the data. Means ± S.E.M. of the aberration frequencies for both the tritium and X-ray treatment groups are plotted on each graph. In addition, means ± S.E.M. of the tritium dose administered to each dose group are also shown on the same graphs. Dicentric and centric ring frequencies are shown in Figure 9, terminal deletion frequencies are shown in Figure

10 and interstitial deletion and acentric ring frequencies are shown in Figure 11. The slope of the aberration frequency with tritium treatment was compared to the slope obtained with X-ray treatment for each group of aberrations. The relative biological effectiveness calculated as a ratio of the two slopes is shown for the different groups of aberrations to be as follows:

	RBE (Tritium/X-rays)	
	from tables	from Appendix XII
Dicentrics and centric rings	1.26	1.14
Terminal deletions	0.93	0.94
Interstitial deletions and acentric rings	1.46	1.49

Photographs of metaphase preparations from peripheral blood lymphocytes are shown in Figures 12 - 16. Figure 12 shows the difference in the staining properties between first and second division metaphases. On the left is a normal first division metaphase. On the right is a second division metaphase showing the differential staining of the two sister chromatids as a result of the fluorescence plus Giemsa staining technique (Perry and Wolff, 1974). This differentiation is useful since only first division metaphases are evaluated for chromosomal aberrations. Some of the typical aberrations observed in peripheral blood lymphocytes are shown in Figures 14 - 16. Aberrations shown in these photographs include dicentrics, terminal deletions,

interstitial deletions, acentric rings, Robertsonian tranlocations, and a chromatid gap.

TABLE 13 Spontaneous Chromosomal Aberrations in the Peripheral Blood Lymphocytes of Unirradiated Control CBA/H Female Mice - Phase II

Prim. Animal Code	Sec. Slide Code	Total Number Of Cells Scored	Number Of Normal Cells	Aberrations									
				Chromosome					Chromatid				
				Dic	Ring	Del	ID	Other	Inter	Iso	Del	ID	Other
51	21	100	100										
52	6	100	97					1 PCD			2		
53	11	100	98										2 gap
54	19	100	99								1		
55	60	100	98					2 PCD					
56	84	100	100										
60	14	100	99					1 PCD					
61	37	100	97				1	2 gap			1		1 gap
63	82	100	98								1		1 gap
64	87	100	97			1	1				1		1 gap
Totals		1000	983			1	2	2 gap 4 PCD			6		5 gap

TABLE 14 Chromosomal Aberrations in the Peripheral Blood Lymphocytes of CBA/H Female Mice Treated with a Nominal 1.5 Gy Dose of Tritiated Water - Phase II

Prim. Animal Code	Sec. Slide Code	Total Number Of Cells Scored	Number Of Normal Cells	Aberrations									
				Chromosome					Chromatid				
				Dic	Ring	Del	ID	Other	Inter	Iso	Del	ID	Other
73	91	60	58			1							1 gap
75	59	100	86	2		11		2 PCD					1 gap
76	30	100	93	1		4		1 trans	1 AI				
77	47	100	84	4		11	1	2 trans			1		
								1 PCD					
78	10	100	82	9	1 ac	8	1	1 trans			4		
79	20	55	48	3		2		1 trans			4		
80	48	85	81			4					1		
81	2	100	87	2	2 ac	4	4	1 trans			1		1 gap
83	51	100	95	4		1					1		
84	55	100	88	10		1		2 trans			1		
85	72	100	84	2		12					6	1	
86	64	100	92	2		4		3 trans					
87	5	100	87	3		7	2	1 trans			1	1	
								1 gap					
88	39	100	87	7		3	3	1 trans					
89	66	100	79	5	1 ac	13	3			1	2		1 gap
90	85	100	83	5		7	1				5		2 gap
91	77	100	91	1		8	1				2		
92	79	100	79	7		9	3	2 trans			3		1 gap
93	1	100	90	2		8			1 SI		4		
94	40	100	83	3		5	3	3 trans	1 SI	1	2		1 gap
								2 PCD					
95	74	100	88	2	1 ac	7		2 trans					
Totals		2000	1745	74	5 ac	130	22	20 trans	2 SI	2	38	2	8 gap
								1 gap	1 AI				
								5 PCD					

TABLE 15 Chromosomal Aberrations in the Peripheral Blood Lymphocytes of CBA/H Female Mice Treated with a Nominal 3.0 Gy Dose of Tritiated Water - Phase II

Prim. Animal Code	Sec. Slide Code *	Total Number Of Cells Scored	Number Of Normal Cells	Aberrations									
				Chromosome						Chromatid			
				Dic	Ring	Del	ID	Other	Inter	Iso	Del	ID	Other
97	94	9	8			1							
98	92	97	79	5		9	3	1 gap		2			1 gap
99	32	54	41			8	3			4			
100	17	100	80	6	1 ac	10	2	1 trans		3			1 gap
101	69	27	26	1	1 ac		1		1 SI				
102	52	37	30	3		4	1			3			1 gap
103	93	28	27			2							
104	62	100	74	5		19	4	1 trans 2 PCD		2	2		
105	90	95	75	5		19	1	1 trans		2	1		1 gap
106	45	97	77	8		13	1	2 trans	2 SI	4			
107	22	100	77	10		13		1 trans 1 PCD					
108	65	100	80	2		15				7	1		1 gap
109	50	100	75	11		12	3	3 trans		8			2 gap
Totals		944	749	56	2 ac	125	19	9 trans 1 gap 3 PCD	3 SI	35	4		7 gap

* Animal Code number 96 (Prim.) , slide #95 (Sec.), was also examined and no mitotic cells were found on any of the four slides prepared.

TABLE 16 Chromosomal Aberrations in the Peripheral Blood Lymphocytes of CBA/H Female Mice Treated with a Nominal 4.5 Gy Dose of Tritiated Water - Phase II

Prim. Animal Code	Sec. Slide Code	Total Number Of Cells Scored	Number Of Normal Cells	Aberrations									
				Chromosome					Chromatid				
				Dic	Ring	Del	ID	Other	Inter	Iso	Del	ID	Other
114	71	50	41	3		4	1				2		2 gap
115	44	50	39	1		8	1	1 trans			3		1 gap
116	68	50	45			3				1	1	1	
117	31	50	45	3		2					3		
118	27	50	42	4		7							
119	3	50	42	2		6				1			
120	75	50	34	9	2 ac	13	2	1 trans			2		
121	80	50	36	8		6	6	1 trans			2	1	
122	76	50	38	11	3 ac	3	4				2	1	
123	18	50	39	17	1 ac	4	1	4 trans			3		
Totals		500	401	58	6 ac	56	15	7 trans		2	18	3	3 gap

TABLE 17 Chromosomal Aberrations in the Peripheral Blood Lymphocytes of CBA/H Female Mice Treated with a Nominal 1.5 Gy Dose of X-rays - Phase II

Prim. Animal Code	Sec. Slide Code	Total Number Of Cells Scored	Number Of Normal Cells	Aberrations									
				Chromosome					Chromatid				
				Dic	Ring	Del	ID	Other	Inter	Iso	Del	ID	Other
128	67	100	90	1		7		1 trans		1			
129	35	100	89	3		6	1	2 trans		1			
130	9	100	94	2		3	1	1 trans		1			
131	86	100	86	1		8				3			5 gap
135	23	100	92	4		6							
136	41	100	87	2		9	1	2 PCD		4			
137	81	100	98			1		1 PCD					1 gap
138	36	100	88	2	2 ac	8	1						
140	29	100	94	2		1		2 PCD		1			
141	46	100	85	1		3	2	4 PCD		4	1		1 gap
								1 trans					
								1 gap					
142	89	100	86	3		10				1	1		
143	61	100	88			6	1	2 PCD			1		1 gap
								1 trans					
144	25 *	100	68	6		6		18 trans		6			3 gap
145	13	100	91	4		3	1	2 gap					
146	88	100	88	2		6	1	1 trans		1			1 gap
147	8	100	89	4		5	1	1 trans		3			
148	53	100	91	4		1	2			1			2 gap
149	16	100	92	1		2	2	2 PCD		1			2 gap
151	7	100	86	4	1 ac	9		1 gap		2			
152	42	100	90	1		7	2			1			
Totals		2000	1772	47	3 ac	107	16	26 trans		31	3		16 gap
								4 gap					
								13 PCD					

* Many translocations were observed on this slide showing the same morphology. The high incidence of this translocation suggests that it may be a karyotypic anomaly rather than the result of radiation-induced chromosomal damage.

TABLE 18 Chromosomal Aberrations in the Peripheral Blood Lymphocytes of CBA/H Female Mice Treated with a Nominal 3.0 Gy Dose of X-rays - Phase II

Prim. Animal Code	Sec. Slide Code	Total Number Of Cells Scored	Number Of Normal Cells	Aberrations									
				Chromosome					Chromatid				
				Dic	Ring	Del	ID	Other	Inter	Iso	Del	ID	Other
156	26	100	93	2		6		1 trans			1		
157	34	100	91			4		2 trans	1 AI		1		1 gap
158	12	100	85	6	1 c	8	1	3 PCD			1		
159	43	100	93	2	1 ac	4	2						
160	28	100	89	3		3		2 trans 2 PCD			2		
163	4	100	82	6		9	1	2 trans		1	2		1 gap
164	78	100	87	3	1 ac	10	1				3		2 gap
165	57	100	82	7		12	3				1	1	1 gap
167	33	100	70	7		15	2	2 trans 1 PCD		1	3		
168	73	100	84	6		8	1	1 trans 1 PCD			3		
Totals		1000	856	42	1 c 2 ac	79	11	10 trans 7 PCD	1 AI	2	17	1	5 gap

TABLE 19 Chromosomal Aberrations in the Peripheral Blood Lymphocytes of CBA/H Female Mice Treated with a Nominal 4.5 Gy Dose of X-rays - Phase II

Prim. Animal Code	Sec. Slide Code	Total Number Of Cells Scored	Number Of Normal Cells	Aberrations									
				Chromosome					Chromatid				
				Dic	Ring	Del	ID	Other	Inter	Iso	Del	ID	Other
172	83	50	43	1		6					2		
173	38	50	45	4		1		1 trans			1		
175	56	50	46	1		2					1		
177	15	50	45			2	2	1 trans 1 PCD					
178	54	50	40	2		7	1	1 trans			1		1 gap
179	24	50	41	3		10	1				2		
180	58	50	38	3		8	2	1 PCD			1		
181	63	50	38	6		6		1 trans			4		
182	49	50	44	2		6		1 trans					
183	70	50	42	4	1 ac	3		1 trans			1		
Totals		500	422	26	1 ac	51	6	6 trans 2 PCD			13		1 gap

TABLE 20 Summary Table of Dicentrics and Acentrics in CBA/H Mouse Peripheral Blood Lymphocytes - Phase II

Dose Group	Number of Cells Scored	Total # of Chromosome-Type Aberrations*		
		Dicentrics + Centric Rings	Terminal Deletions	Interstitial Deletions and Acentric Rings
Control	1000	0	1 (0.10 ± 0.10)	2 (0.20 ± 0.13)
Tritium				
1.5 Gy	2000	74 (3.52 ± 0.60)	130 (6.33 ± 0.78)	27 (1.24 ± 0.37)
3.0 Gy	944**	56 (4.96 ± 1.04)	125 (12.0 ± 1.42)	21 (2.37 ± 0.65)
4.5 Gy	500	58 (11.6 ± 3.38)	56 (11.2 ± 2.05)	21 (4.20 ± 1.67)
X-rays				
1.5 Gy	2000	47 (2.35 ± 0.36)	107 (5.35 ± 0.64)	19 (0.90 ± 0.20)
3.0 Gy	1000	43 (4.30 ± 0.82)	79 (7.90 ± 1.21)	13 (1.30 ± 0.37)
4.5 Gy	500	26 (5.20 ± 1.12)	51 (10.2 ± 1.87)	7 (1.40 ± 0.52)
Slopes from weighted linear regressions				
Tritium		0.01987	0.02924	0.00727
X-rays		0.01582	0.03131	0.00499
Tritium/X-rays		1.26	0.93	1.46

* The numbers within parentheses represent the mean ± S.E.M. of the aberrations per 100 cells (frequency).

** Only 944 cells were scored instead of 1000 because no more cells could be found on any slides from all the mice treated at this dose.

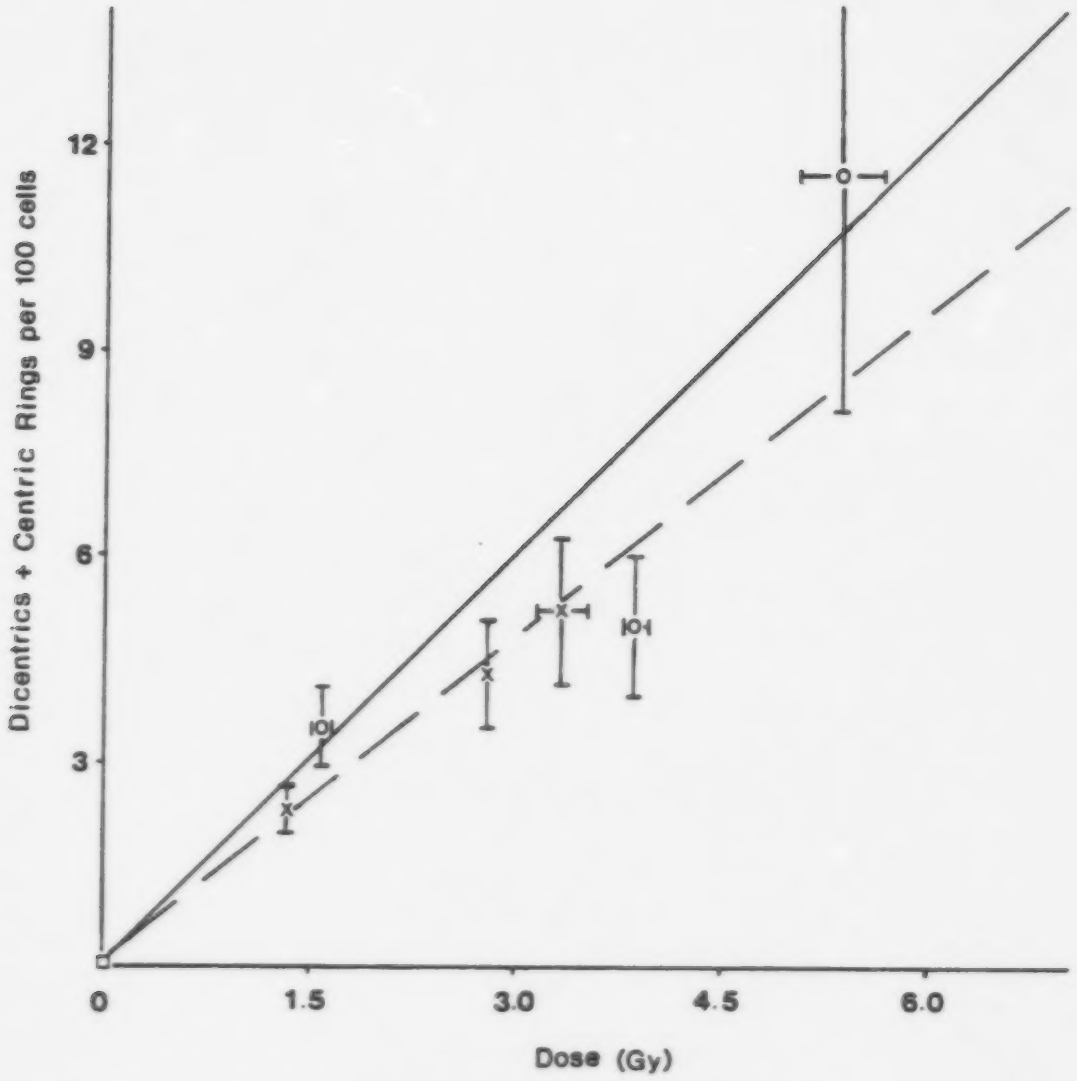

Figure 9. Frequency (mean ± S.E.M.) of dicentrics and centric rings in CBA/H mouse peripheral blood lymphocytes treated with tritium (HTO) and X-irradiation with the weighted linear regression lines - Phase II chromosome aberration study

Legend: Spontaneous frequency: □
Tritium: o, solid line; X-rays: x, dashed line
Vertical error bars indicate the S.E.M. of the frequency. Horizontal error bars indicate the S.E.M. of the radiation dose.

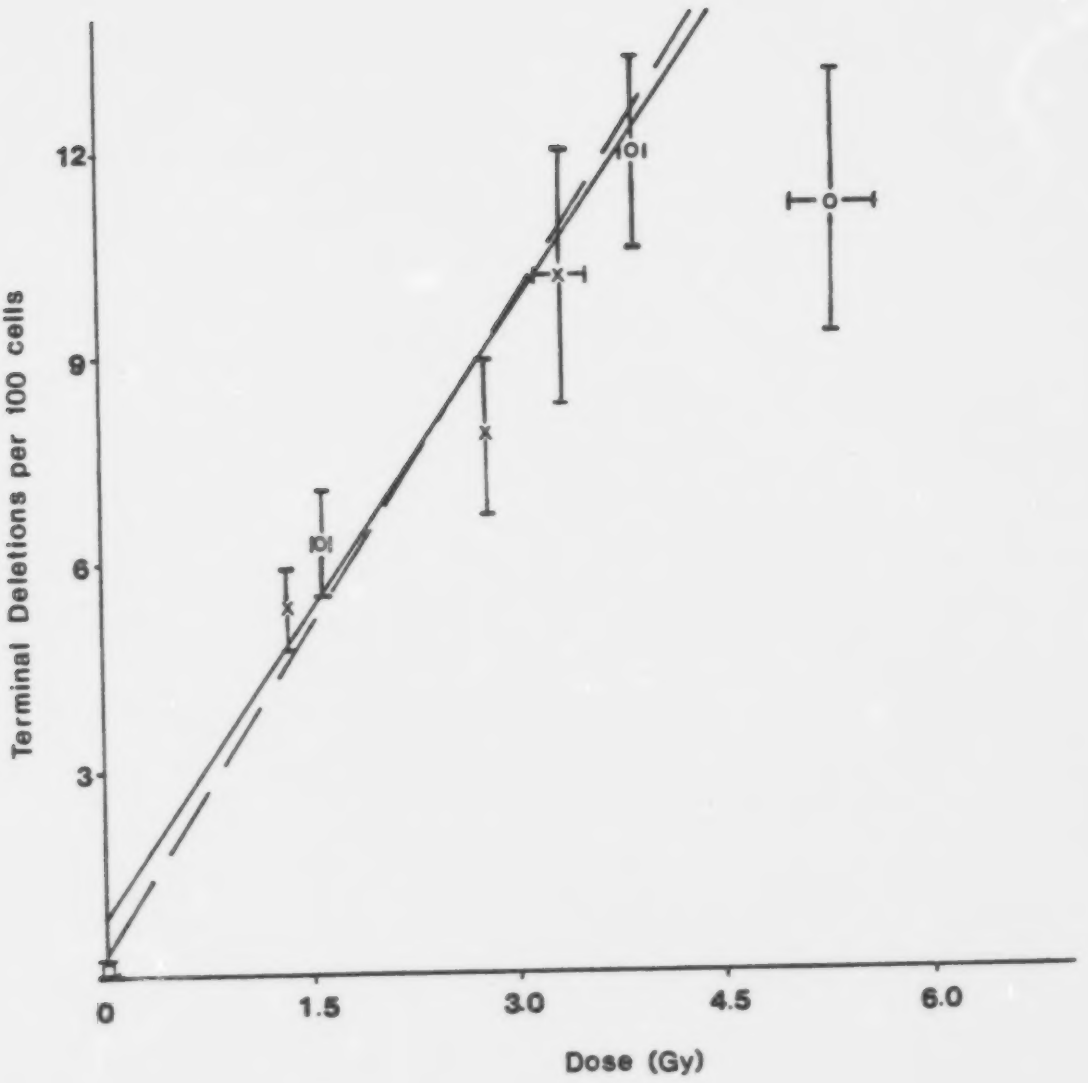

Figure 10. Frequency (mean ± S.E.M.) of terminal deletions in CBA/H mouse peripheral blood lymphocytes treated with tritium (HTO) and X-irradiation with the weighted linear regression lines - Phase II chromosome aberration study

Legend: Spontaneous frequency: □
Tritium: o, solid line; X-rays: x, dashed line
Vertical error bars indicate the S.E.M. of the frequency. Horizontal error bars indicate the S.E.M. of the radiation dose.

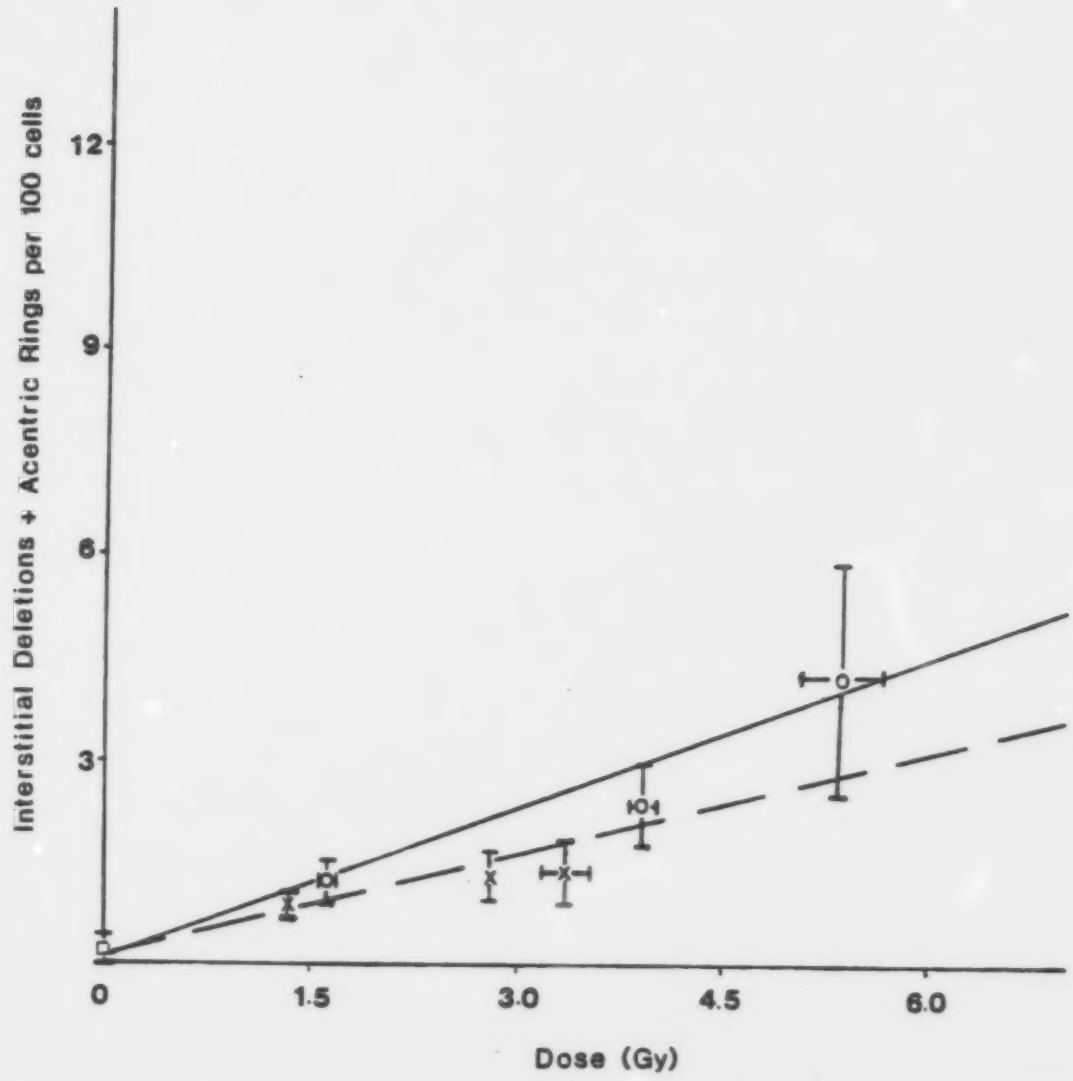

Figure 11. Frequency (mean ± S.E.M.) of interstitial deletions and acentric rings in CBA/H mouse peripheral blood lymphocytes treated with tritium (HTO) and X-irradiation with the weighted linear regression lines - Phase II chromosome aberration study

Legend: Spontaneous frequency: □
Tritium: o, solid line; X-rays: x, dashed line
Vertical error bars indicate the S.E.M. of the frequency. Horizontal error bars indicate the S.E.M. of the radiation dose.

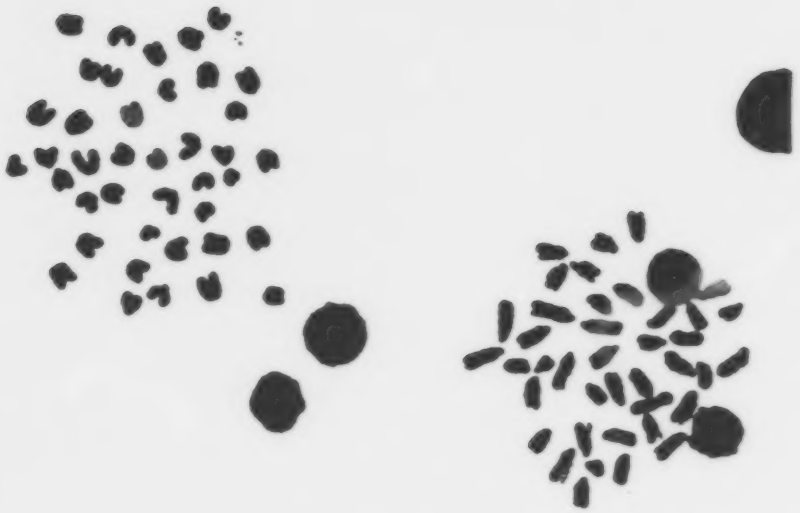

Figure 12. Metaphase cells of CBA/H mouse peripheral blood lymphocytes; left: a normal first division cell; right: a normal second division cell (1000 X)

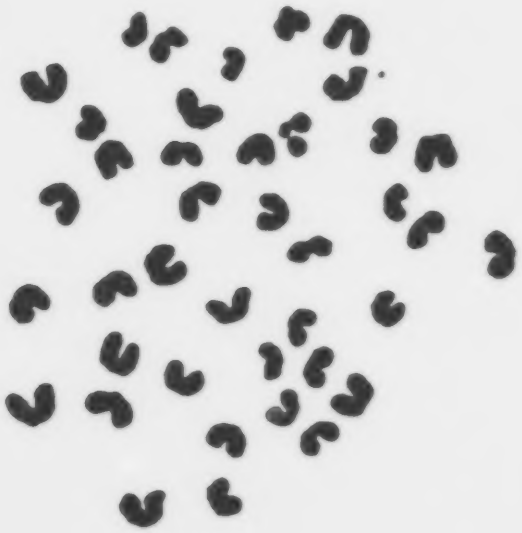

Figure 13. A normal first division metaphase from a CBA/H mouse periperal blood lymphocyte (1000 X)

Figure 14. A metaphase cell from a CBA/H mouse peripheral blood lymphocyte showing a dicentric and associated fragment, a terminal deletion and a chromatid gap (1000 X)

Figure 15. A metaphase cell from a CBA/H mouse peripheral blood lymphocyte showing a dicentric and associated fragment, an interstitial deletion and acentric rings (1000 X)

Figure 16. A metaphase cell from a CBA/H mouse peripheral blood lymphocyte showing a Robertsonian translocation (seen as a metacentric chromosome and a heterochromatic fragment) and an interstitial deletion (1000 X)

C. Effect of Tritium and X-rays on the Induction of Translocationsin the Primary Spermatocytes of the CBA/H Mouse for Phase II

Male CBA/H mice were used for evaluation of translocations in primary spermatocytes and were sacrificed at the rate of approximately 15 mice per day starting at 77 days after initiation of treatment and ending at 86 days. At the time of harvest, during the first few days we observed that there appeared to be a radiation dose-related decrease in testicular size. This led to the weighing and recording of the weight of each testes pair from each animal from day 83 onwards. The results of these weights are shown in Appendix VII. The weights of the testes (pair) were reduced with treatment and are listed below:

	Theoretical Dose	Actual Dose	% Reduction in Weight
Tritium	1.5 Gy	1.43 Gy	15.4
	3.0 Gy	2.71 Gy	50.8
	4.5 Gy	4.34 Gy	67.6
X-rays	1.5 Gy	1.33 Gy	14.3
	3.0 Gy	2.82 Gy	35.7
	4.5 Gy	3.36 Gy	58.4

It should be noted that the actual tritium dose for the heritable translocation portion of the study deviated less from the desired theoretical doses than was observed for the chromosomal aberrations study.

The full spectrum of translocations and other aberrations are listed in Tables 21 - 27 for all the individual animals

in each of the following dose groups:

Table 21	:	controls,
Table 22	:	1.5 Gy tritium,
Table 23	:	3.0 Gy tritium,
Table 24	:	4.5 Gy tritium,
Table 25	:	1.5 Gy X-rays,
Table 26	:	3.0 Gy X-rays and
Table 27	:	4.5 Gy X-rays.

As stated earlier, individual dosimetry data was provided by Atomic Energy of Canada Limited for the individual animals and is shown in Appendix XI. The animals used for the Phase II translocation assay ranged in weight at the different dose levels as follows:

Nominal Dose (Gy)	Weight Range (g)	Mean ± S.E.M.
1.5	20.4 - 24.3	22.0 ± 0.42
3.0	18.1 - 22.9	21.5 ± 0.47
4.5	19.5 - 23.4	21.3 ± 0.35

The tritium dose administered as tritiated water to the animals of each of the three dose groups were as described for the Phase II lymphocyte portion of this study. The tissue dose to each animal of the tritium dose groups ranged as follows:

Nominal Dose (Gy)	Dose Range (Gy)	Mean ± S.E.M.
1.5	1.19 - 1.77	1.43 ± 0.06
3.0	2.40 - 3.16	2.71 ± 0.07
4.5	3.90 - 5.18	4.34 ± 0.11

The results of the heritable translocation assay are shown in Tables 21 - 27. These results are similar to the findings of the Phase II lymphocyte study in that the animals exposed to tritium had a higher frequency of translocations than those exposed to X-rays. A summary of all the translocations noted in this study are shown in Table 28. The total numbers of rings, chains and the combined totals of rings and chains are shown in this table. Table 29 shows the summary of both the X-Y and autosomal univalent types of aberrations. As can be seen in these tables, very few types of aberrations other than those noted above were observed in this study. The category of total translocations was used to calculate the relative biological effectiveness of tritium in comparison to X-rays.

The data show that the number of ring translocations as well as the number of chain translocations are induced to a greater extent by tritium as compared to X-rays. In contrast, no dose-response was noted for the induction of either of the two types of univalents by tritium or X-rays. Individual dosimetry data and number of translocations per animal were used to perform a weighted linear regression analysis.

Figure 17 shows a graphical representation of the data for the total number of translocations observed per dose group. Means $\pm$ S.E.M. of the aberration frequencies for both the tritium and X-ray treatment groups are plotted on each graph. In addition, means $\pm$ S.E.M. of the tritium dose

administered to each dose group are also shown on the same graphs. The slope of the translocation frequency with tritium treatment was compared to the slope obtained with X-ray treatment.

The relative biological effectiveness calculated as a ratio of the two slopes was found to be 1.52.

Table 30 shows the summary of the RBE values for tritium in comparison to X-rays using both chromosomal aberrations as well as translocations as the biological end-points.

Photographs of meiotic preparations from primary spermatocytes are shown in Figures 18 - 22. Figure 18 shows a normal meiotic metaphase preparation. Figures 19 - 22 show the types of aberrations observed in this study. The range of aberrations include autosomal univalents, X-Y univalents, CIV chain configurations, and RIV ring configurations.

TABLE 21 Spontaneous Reciprocal Translocations in the Primary Spermatocytes of Unirradiated Control Male CBA/H Mice

Prim. Animal Code	Sec. Slide Code	Total # of Cells Scored	# of Normal Cells	Rings	Chains			Univalent		Del	Chd	Ex
				RIV	CIII+I	CIV	CVI	X-Y	Autosomal			
184	111	200	147					35	20			
185	134	200	129					70	10	1	1	
187	147	200	149					31	29			
188	102	200	143					31	28	3		
189	128	200	155					26	15	3	1	
190	142	200	149			1		32	13	6		
191	153	200	138					40	27			
193	115	200	147					46	13			
194	159	200	145					35	22			
196	166	200	157		1			39	6			
199	123	200	139					51	18	1		
200	172	200	146	1				42	9	6		
202	162	200	148					39	14	1	1	
203	109	200	144			1		40	17	1		
204	135	200	139					40	23	3		
Totals		3000	2175	1	1	2		597	264	25	3	
Frequency (per 100 cells)			72.5	0.03	0.03	0.07		19.9	8.25	0.84	0.10	
S.E.M.			0.90	0.03	0.03	0.05		1.35	1.07	0.27	0.06	

TABLE 22 Reciprocal Translocations in the Primary Spermatocytes of Male CBA/H Mice Treated with a Nominal 1.5 Gy Dose of Tritiated Water

Prim. Animal Code	Sec. Slide Code	Total # of Cells Scored	# of Normal Cells	Rings	Chains			Univalent		Del	Chd	Ex
				RIV	CIII+I	CIV	CVI	X-Y	Autosomal			
205	143	200	137			3		50	14	2		
206	126	200	137					48	19			
207	117	200	151			1	1	39	10			
208	116	200	150	1		1		33	16	1		
211	160	200	150					41	12			
213	105	200	138	1	2		1	51	14	1		
215	129	200	141			2		36	28			
216	154	200	141					43	19	1		
217	124	200	145	1		1		38	18	3		
218	167	200	145			2		38	15	1		
Totals		2000	1435	3	2	10	2	417	165	9		
Frequency (per 100 cells)			71.8	0.15	0.10	0.50	0.10	20.9	8.25	0.45		
S.E.M.			0.87	0.08	0.10	0.17	0.07	0.97	0.79	0.16		

TABLE 23 Reciprocal Translocations in the Primary Spermatocytes of Male CBA/H Mice Treated with a Nominal 3.0 Gy Dose of Tritiated Water

Prim. Animal Code	Sec. Slide Code	Total # of Cells Scored	# of Normal Cells	Rings	Chains			Univalent		Del	Chd	Ex
				RIV	CIII+I	CIV	CVI	X-Y	Autosomal			
219	136	150	107			1		33	11	3		
221	170	150	110	1	1	1		28	9	1		
222	110	150	97	1		4		40	8	3		
225	173	150	109			3		25	16			
227	141	150	101	3		8		26	20	1		
228	161	150	94	2	2	2		39	19			
229	130	150	86			2		55	8	3		
230	149	150	94	1		2		50	5	1		1
231	125	150	105	6		3		26	12	1		
232	104	150	95	3	2	2		39	10	1		
Totals		1500	998	17	5	28		361	118	14		1
Frequency (per 100 cells)			66.5	1.13	0.33	1.87		29.1	7.87	0.93		0.07
S.E.M.			1.66	0.40	0.18	0.43		2.21	1.05	0.25		0.07

TABLE 24 Reciprocal Translocations in the Primary Spermatocytes of Male CBA/H Mice Treated with a Nominal 4.5 Gy Dose of Tritiated Water

Prim. Animal Code	Sec. Slide Code	Total # of Cells Scored	# of Normal Cells	Rings	Chains			Univalent		Del	Chd	Ex
				RIV	CIII+I	CIV	CVI	X-Y	Autosomal			
233	138	64	55					6	3			
234	176	58	42			1		12	3			
235	177	40	30	1				7	5			
236	178	11	5	2				3		1		
237	179	33	27					6	2			
238	101	55	37	2		3		11	4			
239	145	26	15	1		1		8	2	1		
240	119	37	21			3		12	2			
241	150	7	6	1								
242	156	86	70					13		3		
243	108	100	71					23	8			
244	164	100	62	4				26	7	3		
245	171	100	54	3		3	1	27	11	3		
246	139	100	66			6		24	10	1		
Totals		817*	561	14		17	1	178	57	12		
Frequency (per 100 cells)			68.8	3.54		2.01	0.07	20.8	6.11	1.89		
S.E.M.			3.36	1.52		0.75	0.07	2.31	1.05	0.69		
Theoretical Poisson Error			1.69	0.27		0.29	0.07	0.95	0.54	0.25		

* Only 817 cells were scored instead of 1000 because no more cells could be found on any slides from all the mice treated at this dose.

TABLE 25 Reciprocal Translocations in the Primary Spermatocytes of Male CBA/H Mice Treated with a Nominal 1.5 Gy Dose of X-rays

Prim. Animal Code	Sec. Slide Code	Total # of Cells Scored	# of Normal Cells	Rings	Chains			Univalent		Del	Chd	Ex
				RIV	CIII+I	CIV	CVI	X-Y	Autosomal			
247	168	200	142			2		50	8	1		
250	174	200	124	1		1		56	18	1		
253	118	200	138	1		2		47	15	1		
254	165	200	127			2		54	30	1		
255	157	200	137		2	4		36	16	7		
256	131	200	129	2		3		52	25			
257	107	200	122	1		1		49	31	3		
258	146	200	115					75	13	1		
259	112	200	139		1			46	16			
260	148	200	117	1		1		67	16	2		
Totals		2000	1290	6	3	16		532	188	17		
Frequency (per 100 cells)			64.5	0.30	0.15	0.80		26.6	9.40	0.85		
S.E.M.			1.52	0.11	0.11	0.20		1.74	1.18	0.33		

TABLE 26 Reciprocal Translocations in the Primary Spermatocytes of Male CBA/H Mice Treated with a Nominal 3.0 Gy Dose of X-rays

Prim. Animal Code	Sec. Slide Code	Total # of Cells Scored	# of Normal Cells	Rings	Chains			Univalent		Del	Chd	Ex
				RIV	CIII+I	CIV	CVI	X-Y	Autosomal			
261	121	150	95	4		2		43	13			
262	151	150	102	1		3		37	10	1		
263	158	150	105					39	6	1		
264	175	150	104		3	2		31	11	1		1
265	132	150	107	1	1			36	5	1		
266	169	150	101					40	10	1		
267	144	150	109			1		31	10	5		
269	106	150	96	1				47	11			
270	163	150	101	1				36	13	1		
272	155	150	94			2	1	40	18	1		
Totals		1500	1014	8	4	10	1	380	107	12		1
Frequency (per 100 cells)			67.6	0.53	0.27	0.67	0.07	25.3	7.13	0.80		0.07
S.E.M.			1.07	0.26	0.21	0.25	0.07	1.05	0.77	0.29		0.07

TABLE 27 Reciprocal Translocations in the Primary Spermatocytes of Male CBA/H Mice Treated with a Nominal 4.5 Gy Dose of X-rays

Prim. Animal Code	Sec. Slide Code	Total # of Cells Scored	# of Normal Cells	Rings	Chains			Univalent		Del	Chd	Ex
				RIV	CIII+I	CIV	CVI	X-Y	Autosomal			
275	152	100	73	1			1	21	5	1		
276	133	100	57	1	1	4		36	6	2		
277	122	100	68	1				26	7	1	1	
278	140	100	73	1				20	6	1		
281	113	100	66					27	8			
282	103	100	79	1	1	1		17	2			
283	120	100	76					18	8			
285	127	100	72			1		17	14			
286	137	100	68		1	3		27	9	2		
287	114	100	50	4		1		44	7	1		
Totals		1000	682	9	3	10	1	253	72	8	1	
Frequency (per 100 cells)			68.2	0.90	0.30	1.00	0.10	25.3	7.20	0.80	0.10	
S.E.M.			2.79	0.38	0.15	0.45	0.10	2.80	0.98	0.25	0.10	

TABLE 28 Comparison of Translocations Produced in CBA/H Mouse Primary Spermatocytes by Tritium and X-rays

Dose Group	Number of Cells Scored	# of Translocations*		
		Rings	Chains	Rings and Chains
Control	3000	1 (0.03 ± 0.03)	3 (0.10 ± 0.06)	4 (0.13 ± 0.06)
Tritium				
1.5 Gy	2000	3 (0.15 ± 0.08)	14 (0.70 ± 0.34)	17 (0.90 ± 0.24)
3.0 Gy	1500	17 (1.13 ± 0.40)	33 (2.20 ± 0.61)	50 (3.33 ± 0.68)
4.5 Gy	817**	14 (3.54 ± 1.52)	18 (2.08 ± 0.82)	32 (5.61 ± 1.50)
X-rays				
1.5 Gy	2000	6 (0.30 ± 0.11)	19 (0.95 ± 0.31)	25 (1.25 ± 0.28)
3.0 Gy	1500	8 (0.53 ± 0.26)	15 (1.01 ± 0.53)	23 (1.53 ± 0.44)
4.5 Gy	1000	9 (0.90 ± 0.38)	14 (1.40 ± 0.70)	23 (2.30 ± 0.67)

Slopes from weighted linear regressions	
Tritium	0.0102
X-rays	0.00669
Tritium/X-rays	1.52

* The numbers within parentheses represent the mean ± S.E.M. of the translocations per 100 cells (frequency).

** Only 817 cells were scored instead of 1000 because no more cells could be found on any slides from all the mice treated at this dose.

TABLE 29 Comparison of the Production of Autosomal and X-Y Univalents in CBA/H Mouse Primary Spermatocytes by Tritium and X-rays

Dose Group	Number of Cells Scored	X-Y Univalents*	Autosomal Univalents*
Control	3000	597 (19.9 ± 1.35)	264 (8.25 ± 1.07)
Tritium			
1.5 Gy	2000	417 (20.9 ± 0.97)	165 (8.25 ± 0.79)
3.0 Gy	1500	361 (29.1 ± 2.21)	118 (7.87 ± 1.05)
4.5 Gy	817**	178 (20.8 ± 2.31)	57 (6.11 ± 1.05)
X-rays			
1.5 Gy	2000	532 (26.6 ± 1.74)	188 (9.40 ± 1.18)
3.0 Gy	1500	380 (25.3 ± 1.05)	107 (7.13 ± 0.77)
4.5 Gy	1000	253 (25.3 ± 2.80)	72 (7.20 ± 0.98)

* The numbers within parentheses represent the mean ± S.E.M. of the translocation per 100 cells (frequency).

** Only 817 cells were scored instead of 1000 because no more cells could be found on any slides from all the mice treated at this dose.

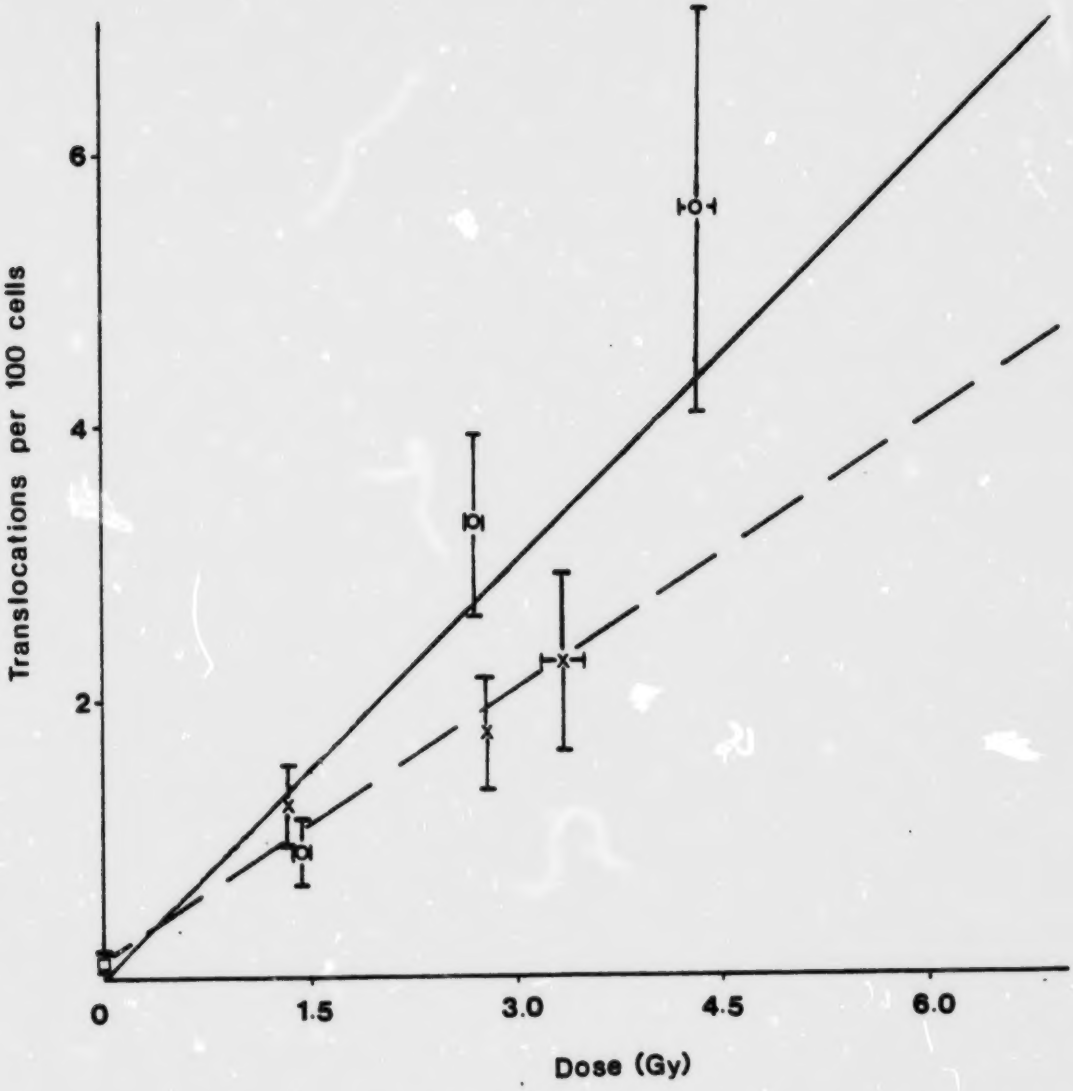

Figure 17. Frequency (mean ± S.E.M.) of total translocations in CBA/H mouse primary spermatocytes treated with tritium (HTO) and X-irradiation with the weighted linear regression lines - Phase II heritable translocation study

Legend: Spontaneous frequency: □
Tritium: o, solid line; X-rays: x, dashed line
Vertical error bars indicate the S.E.M. of the frequency. Horizontal error bars indicate the S.E.M. of the radiation dose.

TABLE 30 Relative Biological Effectiveness (RBE) of Tritium in Comparison to X-rays Derived from the Induction of Dicentrics and Centric Rings in the Peripheral Blood Lymphocytes and Translocations in the Primary Spermatocytes of the CBA/H Mouse

Aberration	Slope of Tritium Curve *	Slope of X-ray Curve *	RBE = Slope of Tritium / Slope of X-rays *
Dicentrics and Centric Rings	0.01987	0.01582	1.26
Translocations	0.0102	0.00669	1.52

* The slopes are defined as the number of aberrations/Gy.

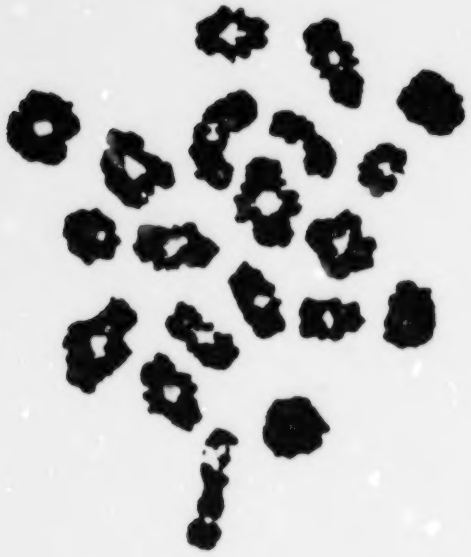

Figure 18. A normal meiotic cell from a CBA/H mouse primary spermatocyte (1000 X)

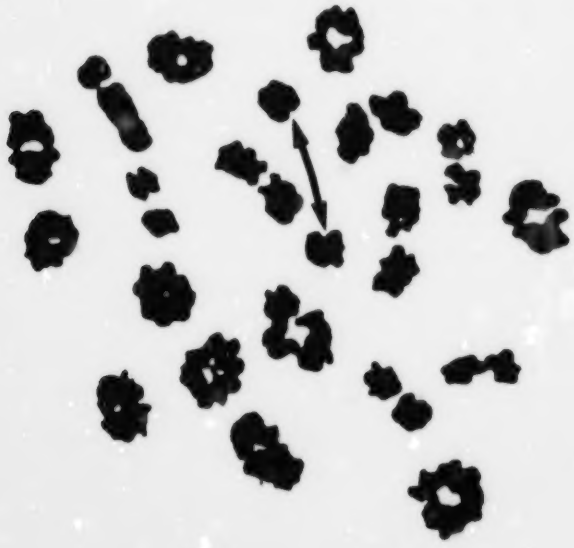

Figure 19. A meiotic cell from a CBA/H mouse primary spermatocyte showing autosomal univalents (1000 X)

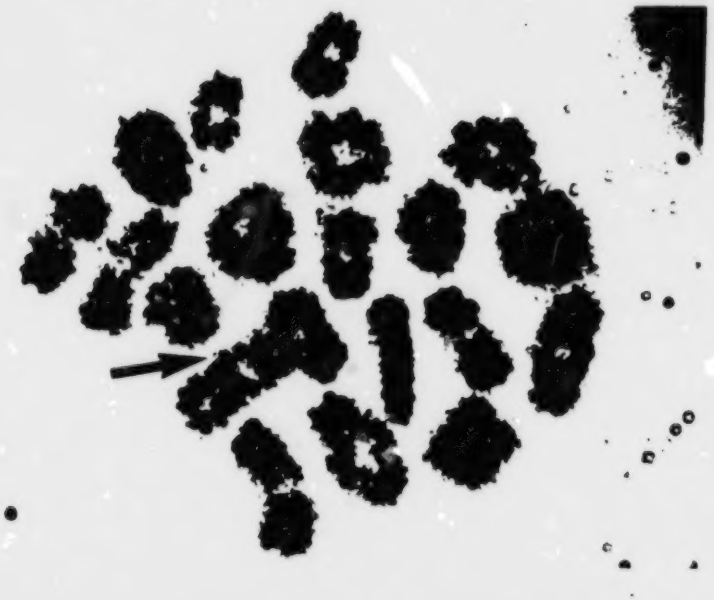

Figure 20. A meiotic cell from a CBA/H mouse primary spermatocyte showing a CIV chain translocation (1000 X)

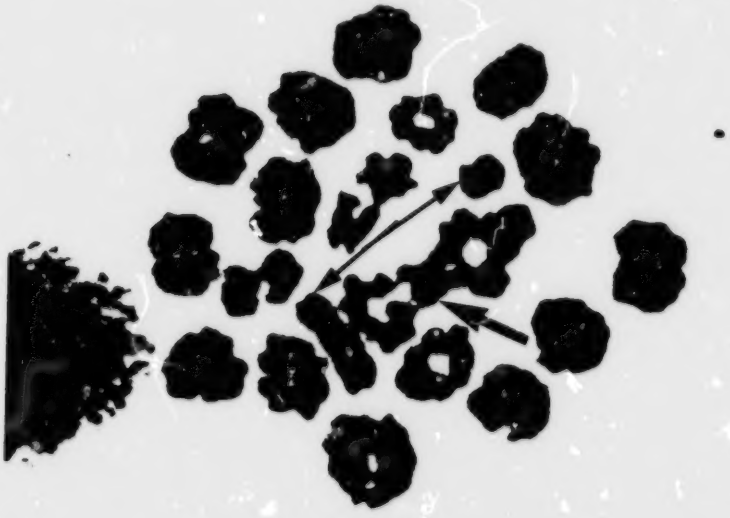

Figure 21. A meiotic cell from a CBA/H mouse primary spermatocyte showing a CIV chain translocation and X-Y univalents (1000 X)

Figure 22. A meiotic cell from a CBA/H mouse primary spermatocyte showing an RIV ring translocation (1000 X)

D. Other Considerations

It is necessary to mention that all the animals in each section of the study were not sacrificed for culture of lymphocytes or preparation of primary spermatocytes on the same day. In order to reduce bias and to minimise any day-to-day variations in procedure all the animals for each dose group were divided equally among the scheduled number of harvest days. Sacrifice for Phase I was performed over a 3-day period with approximately 14 animals sacrificed each day. Phase II for lymphocytes and spleen cell culture was carried out over a 7-day period with approximately 19 animals sacrificed each day. Phase II for spermatocyte preparation was performed over a 10-day period with an interruption of 3 days in between. Approximately 15 animals were sacrificed each day.

DISCUSSION

The aim of this study was to determine the relative biological effectiveness of tritium in comparison to 250 kVp X-rays for use in biological radiation dosimetry and in risk estimation. For this reason, chronic exposure was an important component of the study design. In this study, the exposure to tritium was initiated by a single intraperitoneal injection of tritiated water. Although the route of administration differs from that which atomic radiation workers experience, regardless of the route of exposure, water is rapidly circulated throughout the body. Since water is rapidly exchanged in the body, the dose decreases exponentially with a half life of approximately 2 to 3 days (J. R. Johnson, personal communication). In order to correspond to this declining chronic exposure, the X-rays were delivered in decreasing daily doses to mimic, as closely as possible, the expected daily doses from tritiated water for the first few days. For details of the methods of X-ray dosing used in this study, see Appendix XIII.

1. RBE for Tritium for the Chromosomal Aberrations of Interest

The chromosomal aberrations of greatest interest in both lymphocytes and spermatocytes are those involving the exchange of chromosomal material within or between chromosomes. Since this must involve breaks in the chromosomes at both chromosomal locations involved, these aberrations can arise from two independently induced chromosomal breaks. Such two-hit aberrations will increase as the square of the dose. In practice, it is found that

these aberrations are also inducible by a single hit with most radiations, the proportion of one- and two-hit aberrations varying with the energy of the radiation and the dose rate. The more densely ionizing the radiation, the greater will be the proportion of one-hit aberrations. All radiations have sufficient densely ionizing components of the energy distribution spectrum to produce some one-hit aberrations. At chronic exposure rates, two-hit aberrations are more rare because some chromosomal lesions are repaired before others are induced, thus reducing the potential for exchange. One-hit aberrations are unaffected by this factor. As a result, the dose-response curve for all aberration types tends to become linear in chronic exposures. This makes the analysis of the results and the comparison of the two radiation types easier, as simple linear regressions can be used to fit the dose-response data. The RBE is then the ratio of the slopes of the two lines of best fit. In this study, larger samples were scored at low doses than high doses to compensate for the lower frequency of chromosomal aberrations expected at the lower doses.

The difficulty of culturing mouse peripheral blood lymphocytes has resulted in a dearth of studies in the literature, especially chronic radiation studies. Nevertheless, there has been considerable work on the relationship between the frequency of dicentrics in cultured lymphocytes of various mammals, including the mouse, and the

frequency of translocations induced in primary spermatocytes. There is a close theoretical relationship between the dicentrics induced in lymphocytes and the translocations induced in spermatocytes. Both involve the exchange of material between two chromosomes. At the site of such an exchange, the only difference between the two aberrations is morphological: if the two centric ends rejoin together, the result is a dicentric whereas if a centric end rejoins with the acentric end of the other chromosome, the result is a translocation. In any given cell type, the dicentrics and translocations are normally formed with equal frequency, but are not equally detectable. In lymphocytes, many of the translocations escape notice because the lengths of chromosomal material exchanged are too similar for the altered shape of the chromosomes to be detected easily. In spermatocytes, a dicentric chromosome and the acentric fragment that inevitably accompanies it are lethal to the cell during divisions that intervene between the induction of the aberration and the meiotic division at which the translocations are scored. Thus, there is reason to expect that the results obtained for dicentric chromosomes in lymphocytes should be similar to those obtained for translocations in spermatocytes, although differences between the two cell types certainly exist that could influence their response to the radiation exposures.

The data obtained in this study show that indeed the two aberration types, dicentrics in lymphocytes and

translocations in spermatocytes, did respond similarly. It should be noted that the lymphocyte data are reported as dicentrics plus centric rings, as is usual in radiation dosimetry, but the meiotic counterparts to centric rings, pericentric inversions, were not scored in spermatocytes as they are not readily detected. There is, however, no meaningful difference between the frequency of dicentrics plus centric rings and that of dicentrics alone, since only one centric ring was observed in the entire Phase II study. Murine chromosomes have their centromere almost at the end of the chromosome, so that there is very little chance of exchange between arms to form a centric ring.

The RBE for dicentric plus ring production in lymphocytes was 1.14; the RBE for translocations in spermatocytes was 1.21. These two values are remarkably similar, given the uncertainties associated with each. In both cases the confidence interval (see Appendix XII) overlaps 1. Perhaps the best overall estimate of the RBE is the average of these two values, i.e., an RBE of 1.18 which would indicate a slightly greater efficiency for tritium.

Prosser and coworkers (1983) found an RBE of 1.1 to 1.2 for tritium in comparison to 250 kVp X-rays (Lloyd et al., 1975 when using the induction of chromosomal aberrations (dicentrics) in human lymphocytes. Our data for these aberrations in mouse peripheral blood lymphocytes are very similar. In both cases, the reference radiation was 250 kVp

X-rays. It should be noted that *in vitro* exposure for a short period of time may differ significantly from protracted *in vivo* exposure. In the *in vivo* exposure, there is more time for the tritium to become distributed throughout the cellular molecules. Obviously there are other possible explanations as well, including other differences between *in vivo* and *in vitro* exposures, other differences in experimental methods, experimental errors, and the species difference. The latter difference, while the most obvious one, is unlikely. Many other studies involving species as different as man and broadbeans have shown that qualitatively the chromosomes of different species respond alike when allowance is made for the different shapes and cellular arrangements of chromosomes.

Most of the previous work has not been done under conditions that permitted the unambiguous identification of first division cells, as was done here. The general effect of this difference should have been to cause an underestimate of the number of aberrations induced at any given dose. If, as our results suggest, there is also a greater mitotic delay from tritium beta rays than from X-rays, this would result in an underestimate of the RBE since the X-ray samples would be more contaminated with second division cells which have lower frequencies of aberrations than would the tritium samples. Nevertheless the results are in excellent agreement.

2. RBE of Tritium for Other Effects

(a) Other Chromosomal Aberrations:

Two other classes of chromosomal aberrations were measured in lymphocytes: (i) terminal deletions and (ii) acentric rings, of which interstitial deletions are a morphological subclass. Terminal deletions had an RBE of 0.9 whereas interstitial deletions and acentric rings had an RBE of 1.5. The acentric rings, which involve breaks in two chromosomal regions, like the dicentrics and translocations, had an RBE close to the average value obtained for the latter two aberration types. It is significant that terminal deletions are the only class of aberrations that involve a single chromosome break. As the distribution of energy deposition events that lead to chromosome aberrations becomes more clustered, the probability that both chromosomes at a potential exchange site will be affected increases even if the total number of such lesions is unchanged, thus producing an increased RBE for such exchanges. Hence, even if the total number of chromosomal lesions that can result in a chromosome break is unchanged, the number of exchanges would be increased. For terminal deletions, however, the distribution of such lesions is irrelevant, as each has some probability of becoming an aberration. If the difference in energy distribution is such that the total number of chromosomal lesions that can give rise to aberrations is much the same between tritium beta rays and 250 kVp X-rays, little difference in the frequency of terminal deletions

will result and the RBE will be about 1 for these aberrations. At the same time, however, the distribution of lesions can differ so that the frequency of exchange aberrations such as dicentrics, translocations, and acentric rings is enhanced. The chromosomal data are thus internally consistent.

(b) Cellular Toxicity:

At the high dose of tritium, the mitotic index was substantially reduced (qualitative observation). In some cases, this reduction did not permit analysis of the desired number of cells per animal. By contrast, X-rays did not appear to be quite so toxic as tritium beta rays, since a sufficient number of mitotic cells could be found for analysis. The total number of spleen cells per animal were also compared. The results (Appendix VI) showed that at all three doses both tritium and X-ray treatment led to a depression in the number of spleen cells recovered. The decrease in total cell number was greater for tritium compared to X-rays and was also seen to be dose related. Another indicator of the toxicity of tritium was the testicular size as defined by the total wet weight of both testes. Once again tritium was seen to be more toxic in comparison to X-rays and both types of radiation resulted in reduced testicular weights in comparison to unirradiated controls. Similar results have been noted in the literature. Carsten and coworkers (1977) noted a depression in the total number of bone marrow stem cells

when evaluating the toxic effects of tritium. Storer and coworkers (1957) noted very similar depressions in spleen and testicular weights as we did in this study.

(c) Chromosomal Aberrations in Phase I:

In Phase I, it was found that the splenic lymphocytes were substantially more sensitive than the peripheral blood lymphocytes as measured by chromosomal damage. This conclusion is not affected by the lack of individual animal dosimetric data in Phase I, because both splenic and peripheral blood lymphocytes were cultured from the same animals. The difference was large, approximately three- to four-fold for tritium and two- to three-fold for X-rays. Since spleen cells were also cultured from Phase II animals, it would be possible to confirm this difference. It could then be determined whether the difference observed between animals is, in fact, a genuine difference in susceptibility that affects at least these two cells types. Furthermore, it would confirm the RBE measurements observed using PBLs. The data also suggest that at low levels of exposure, splenic cells are a much more sensitive indicator compared to blood lymphocytes. Nevertheless, the sample sizes were small and the conclusions are therefore tentative.

Since splenic lymphocytes were cultured from the same animals, analysis of these cells could confirm or deny the existence of inherent genetic differences in susceptibility among the animals used in this experiment. In addition,

because of the greater sensitivity of the splenic lymphocytes, a larger number of aberrations would be seen, and thus a more reliable estimate of the RBE would be obtained.

Because the nominal and actual doses in Phase II differed substantially, and only nominal doses were available for Phase I, any comparison between Phases I and II is dubious. Given this uncertainty, the results of the two phases are not inconsistent.

3. X-Y Univalents

The frequency of X-Y univalents ranged from about 20 to 29 percent in all the groups and seemed to be somewhat higher than the approximately 10% value cited in the literature (Leonard, 1973). This may simply be due to a strain difference in baseline levels or, alternatively, an artifact due to our particular method of cell and slide preparation. The formation of autosomal univalents similarly showed no correlation with radiation dose or type and ranged from about 7 to 8 percent in all cases.

4. Extrapolation to Specific Locus Mutations

It has long been known that there is a strong parallel between the results of the specific locus mutation assay (Russell, 1951) for gene mutations in the mouse and spermatogonial translocations. The infertility of many specific locus mutations when homozygous suggested that a

significant fraction were small deletions and thus comparable to chromosomal aberrations detected cytogenetically. Molecular analysis has confirmed this suspicion. Hence it is to be expected that the RBE for specific locus mutations is similar to the RBE for translocations.

5. Other Estimates of the RBE for Tritium

The RBE of tritium varies with the dose rate (Ujeno, 1983). The lower dose rates often show considerably higher RBEs, for example, an RBE of 3 to 4, as measured by mouse oocyte survival (Dobson and Kwan, 1976). The selection of inappropriate standards results in another source of variation and confusion (Carsten et al., 1981). RBE values range from as low as 1 when low energy X-rays serve as the reference source to as high as 3 when cobalt-60 gamma rays are compared. The current dilemma emanates from the fact that each study seems to utilise a broad range of radiation types and dose rates. In addition, the range of end points used also show a range of RBE values (Ise et al., 1984, Vennart, 1968, Ueno et al., 1982). It should be noted, of course, that it is the RBE values obtained from low dose radiation which most closely resemble chronic exposures. An in-depth review of tritium toxicity and dosimetry will soon be published by the Atomic Energy Control Board (Myers and Johnson, in press).

It seems to be generally accepted that the RBE of tritium is

1.0 in comparison to reference radiation such as X-rays or gamma rays (Johnson et al., 1986a, Myers and Johnson, in press). There are, however, widely ranging RBE values for tritium reported in the literature which vary with the end point measured. Carsten (1979) quotes a re-evaluation of the data of several investigators by Johnson (1973) where the RBE of tritium ranges from somewhat greater than 1 to lower than 3. Some of the end points used in the different studies include HeLa cell growth rate (Hall et al., 1967), thymic or splenic atrophy (Storer et al., 1957), injury to mouse spermatogonia (Lambert, 1969) and mouse oocyte survival (Dobson and Kwan, 1976).

Biologically significant end-points such as induction of cancer and genetic effects have not been extensively examined (Johnson et al., 1986b). Gragtmans and coworkers (1984) studied the effect of tritium beta rays in comparison to 200 kVp X-rays for their ability to accelerate the appearance of mammary tumors in Sprague-Dawley rats. They found an RBE ranging from 1.0 to 1.4. A comprehensive study is currently underway to examine the effect of tritium beta rays upon the induction of myeloid leukemia in CBA/H mice (Johnson et al., 1986b). This study should help to define a more appropriate RBE for tritium in comparison to 250 kVp X-rays, especially when considered in conjunction with the RBE values obtained from the genetic end points examined in the present study. In addition, the myeloid leukemia study should provide more useful direct information regarding the

relative oncogenic potential of tritium. The present study also provides useful information to this end, since the determinative end points being used to evaluate genetic damage in this study, are often indicative of risk for cancer, heritable genetic disorders and sterility or semi-sterility.

CONCLUSIONS

The RBE for chronic exposure to tritium via tritiated water in comparison to 250 kVp equivalent X-rays (150 kVp X-rays suitably filtered) is estimated to be 1.2 for cytogenetic effects with a rather wide range of uncertainty. An RBE of 1 cannot be excluded by the data. For the dicentric and ring chromosomes used for biological dosimetry, the RBE observed was 1.14. For the translocations which contribute to genetic risk the RBE observed was 1.21.

The findings of the first part of this study indicate that spleen cells are more sensitive (2- to 4-fold) indicators of radiation-induced aberrations, in addition to being much easier to culture and yielding large numbers of mitoses. It is recommended that an analysis of the aberration frequencies in splenic lymphocytes, which have already been prepared for chromosomal analysis from the same animals, be conducted. This analysis would confirm or deny the differences in animal to animal sensitivity observed in the peripheral lymphocytes and, because of the higher aberration frequency, would permit a more precise determination of the RBE. It would also be possible to increase the sample size for many of the peripheral lymphocyte and spermatogonial samples already scored, particularly at the lower dose in order to reduce the uncertainties associated with the estimated RBE.

The results of this study suggest that the effect of tritium on cytogenetic effects is unlikely to differ much from that of an equivalent dose of X-rays, both somatically and genetically, although unfortunately the range of possible values is quite wide.

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APPENDICES

Note for all the Appendices

It should be noted that all the doses in the appendices are written as the theoretical doses of 2.0 and 6.0 Gy or 1.5, 3.0 and 4.5 Gy. In Phase I, where no dosimetry data are available, these are the only doses which may be written. In the Phase II chromosomal aberrations study, the actual doses are noted as follows:

Theoretical Dose	Actual Mean Dose	
	Tritium	X-rays
1.5 Gy	1.52 Gy	1.33 Gy
3.0 Gy	3.87 Gy	2.82 Gy
4.5 Gy	5.36 Gy	3.36 Gy

In the Phase II heritable translocations study, the actual doses are noted as follows:

Theoretical Dose	Actual Mean Dose	
	Tritium	X-rays
1.5 Gy	1.43 Gy	1.33 Gy
3.0 Gy	2.71 Gy	2.82 Gy
4.5 Gy	4.34 Gy	3.36 Gy

APPENDIX I

ANIMAL CODE SHEET Page #: 1

Phase I / Phase II / Spermatocytes (circle one)

Mouse strain: CBA/H Sex: male / female (circle one)

Treatment Group	Mouse Number	Status / Cell Culture Date
Control	1	
	2	
	3	cultured on Feb 23/87
	4	
	5	
	6	
	7	
	8	Feb 24/87
	9	
	10	
	11	
	12	
	13	Feb 25/87
	14	
	15	
Tritium 2 Gy	16	Feb 23/87
	17	Feb 23/87
	18	Feb 24/87
	19	Feb 24/87
	20	Feb 24/87
	21	Feb 25/87

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Treatment Group	Mouse Number	Status / Cell Culture Date
	22	Feb 25/87
	23	Feb 25/87
Tritium 6 Gy	24	found dead on feb 17/87
	25	found dead on feb 17/87
	26	Cultured on feb 17/87
	27	Cultured on feb 17/87
	28	Feb 22/87
	29	Feb 24/87
	30	Blood withdrawal failed on Feb 25/87
	31	Feb 25/87
Gamma rays 2 Gy	32	Feb 23/87
	33	
	34	
	35	Feb 24/87
	36	Feb 24/87
	37	Feb 25/87
	38	
	39	
Gamma rays 6 Gy	40	Feb 23/87
	41	
	42	
	43	Feb 24/87
	44	Feb 24/87

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Treatment Group	Mouse Number	Status / Cell Culture Date
	45	Feb. 24/87
	46	Feb. 24/87
	47	Feb. 25/87

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EAR TAG CODE

Animals treated with tritiated water were identified by an ear hole-punching code for the Phase II lymphocyte and spermatocyte phase of the project. Animals treated with tritium were weighed and individually dosed to enable correlation of body weight with cytogenetic data.

The ear hole-punching code is deciphered as follows:

Animal #	Hole-Punch Code
1	entire hole in the "top" of the ear
2	entire hole in the "middle" of the ear
3	entire hole in the "bottom" of the ear
4	semi-circle in the "top" of the ear
5	semi-circle in the "middle" of the ear
6	semi-circle in the "bottom" of the ear
7	two semi-circles in the "top" of the ear

ANIMAL CODE SHEET Page #: 1

Phase I / (Phase II) / Spermatocytes (circle one)

Mouse strain: CBA/H Sex: male / (female) (circle one)

Treatment Group	House Number	Status / Cell Culture Date
Control	48	
	49	May 5/87
	50	
	51	
	52	May 6/87
	53	
	54	
	55	May 7/87
	56	
	57	
	58	May 8/87
	59	
	60	
	61	May 9/87
	62	
	63	
	64	May 10/87
	65	
	66	
	67	May 11/87
	68	

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Treatment Group	Mouse Number	Status / Cell Culture Date
Tritium 1.5 Gy	69 8	
	70 6	
	71 7	May 5/87
	72 1	HTO-C
	73 5	
	74 4	May 6/87
	75 2	
	76 2	
	77 3	
	78 7	HTO-D
	79 1	May 7/87
	80 6	
	81 4	
	82 5	May 8/87
	83 3	
	84 5	
	85 6	HTO-E
	86 2	May 9/87
	87 1	
	88 7	
	89 1	
	70 3	HTO-F May 10/87
	91 5	

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Treatment Group	House Number		Status / Cell Culture Date
	92	6	May 10/87
	93	4	
	94	2	HTO-F — May 11/87
	95	7	
Tritium 3.0 Gy	96	5	May 5/87
	97	~~2~~ 1	
	98	6	HTO-I — May 6/87
	99	2	
	100	4	May 7/87
	101	7	
	102	~~3~~ 3 6th	May 8/87
	103	6	
	104	~~7~~ 7 12th 9 May	May 9/87
	105	~~4~~ 4	HTO-J
	106	5	May 10/87
	107	~~3~~ 3 9th 10 May	
	108	2	May 11/87
	109	1	
Tritium 4.5 Gy	110	4	May 5/87
	111	3	
	112	2	HTO-M — May 6/87
	113	5	
	114	7	May 7/87

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Treatment Group	House Number	Status / Cell Culture Date
	115 1	HTO-H cont'd — May 7/87
	116 6	May 8/87
	117 4	
	118 1	May 9/87
	119 6	HTO-N
	120 7	May 10/87
	121 2	
	122 5	May 11/87
	123 3	
X-ray 1.5 Gy	124	
	125	May 5/87
	126	
	127	
	128	
	129	May 6/87
	130	
	131	
	132	
	133	May 7/87
	134	
	135	
	136	May 8/87
	137	May 8/87

Treatment Group	House Number	Status / Cell Culture Date
	139	May 8/87
	139	May 8/87
	140	
	141	May 9/87
	142	
	143	
	144	
	145	May 10/87
	146	
	147	
	148	
	149	
	150	
	151	May 11/87
	152	
	153	
x-ray 3.0 Gy	154	
	155	May 5/87
	156	
	157	May 6/87
	158	
	159	May 7/87
	160	May 8/87

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contd.

Treatment Group	Mouse Number	Status / Cell Culture Date
	161	May 8/87
	162	
	163	May 9/87
	164	
	165	May 10/87
	166	
	167	May 11/87
	168	
X-ray 4.5 Gy	169	May 5/87
	170	
	171	May 6/87
	172	
	173	May 7/87
	174	
	175	May 8/87
	176	
	177	May 9/87
	178	
	179	May 10/87
	180	
	181	
	182	May 11/87
	183	

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ANIMAL CODE SHEET Page #: 1

Phase I / Phase II / Spermatocytes (circle one)

Mouse strain: CBA/H Sex: male / female (circle one)

Treatment Group	Mouse Number	Status / Cell Culture Date
Control	184	
	185	June 22/67
	186	
	187	
	188	June 23/67
	189	
	190	
	191	June 24/67
	192	
	193	
	194	June 25/67
	195	
	196	
	197	June 26/67
	198	
	199	
	200	June 30/67
	201	
	202	
	203	July 1/67
	204	

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Treatment Group	Mouse Number	Status / Cell Culture Date
Tritium 1.5 Gy	205	HTO-B
	206	HTO-A
	207	HTO-A
	208	HTO-B
	209	HTO-B
	210	HTO-B
	211	HTO-B
	212	HTO-B
	213	HTO-B
	214	HTO-A
	215	HTO-A
	216	HTO-A
	217	HTO-A
	218	HTO-A
Tritium 3.0 Gy	219	HTO-H
	220	HTO-G
	221	HTO-G
	222	HTO-H
	223	HTO-H
	224	HTO-H
	225	HTO-H
	226	HTO-H
	227	HTO-H

Treatment Group	House Number	Status / Cell Culture Date
	228 #2 HTO-G	June 21/87
	229 #6 HTO-G	June 21/87
	230 #4 HTO-G	
	231 #7 HTO-G	July 1/87
	232 #3 HTO-G	
Tritium 4.5 Gy	233 #5 CAGE HTO-L	June 11/87
	234 #6 HTO-K	June 22/87
	235 #7 HTO-K	June 23/87
	236 #1 HTO-L	
	237 #5 HTO-K	June 24/87
	238 #3 HTO-K	
	239 #4 HTO-K	June 25/87
	240 #2 HTO-K	
	241 #1 HTO-K	June 26/87
	242 #8 HTO-L	
	243 #6 HTO-L	June 27/87
	244 #1 HTO-L	
	245 #4 HTO-L	July 1/87
	246 #7 HTO-L	
X-ray 1.5 Gy	247	June 22/87
	248	
	249	June 23/87
	250	

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Treatment Group	Mouse Number	Status / Cell Culture Date
	251	June 24/57
	252	"
	253	June 25/57
	254	
	255	June 26/57
	256	
	257	June 30/57
	258	
	259	July 1/57
	260	
X-ray 3.0 Gy	261	June 22/57
	262	
	263	June 23/57
	264	
	265	June 24/57
	266	
	267	June 25/57
	268	
	269	June 26/57
	270	
	271	June 30/57
	272	
	273	July 1/57

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Treatment Group	Mouse Number	Status / Cell Culture Date
	274	July 1/87
X-ray 4.5 Gy	275	June 16/87
	276	June 22/87
	277	June 25/87
	278	
	279	June 24/87
	280	
	281	June 25/87
	282	
	283	June 26/87
	284	
	285	June 30/87
	286	
	287	July 1/87
	288	Died on June 15/87

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APPENDIX II

Phase I Schedule
Animal Utilization - February 23 - 25, 1987

Treatment Group	Monday February 23	Tuesday February 24	Wednesday February 25
Control	1-5	6-10	11-15
Tritium			
2.0 Gy	16-17	18-20	21-23
6.0 Gy	28	29	30-31
X-Rays			
2.0 Gy	32-34	35-36	37-39
6.0 Gy	40-42	43-45	46-47

PHASE II SCHEDULE

Animal Utilisation - May 5 - 11, 1987

Treatment Group	Tuesday May 5	Wednesday May 6	Thursday May 7	Friday May 8
Control	48-50	51-53	54-56	57-59
Tritium				
1.5 Gy	69-72	73-76	77-80	81-84
3.0 Gy	96-97	98-99	100-101	102-103
4.5 Gy	110-111	112-113	114-115	116-117
X-Rays				
1.5 Gy	124-127	128-131	132-135	136-139
3.0 Gy	154-155	156-157	158-159	160-161
4.5 Gy	169-170	171-172	173-174	175-176

Treatment Group	Saturday May 9	Sunday May 10	Monday May 11
Control	60-62	63-65	66-68
Tritium			
1.5 Gy	85-88	89-92	93-95
3.0 Gy	104-105	106-107	108-109
4.5 Gy	118-119	120-121	122-123
X-Rays			
1.5 Gy	140-143	144-147	148-153
3.0 Gy	162-163	164-165	166-168
4.5 Gy	177-178	179-180	181-183

SPERMATOCYTE SCHEDULE
Animal Utilisation - JUNE 22 - JULY 1, 1987

Treatment Group	Monday June 22	Tuesday June 23	Wednesday June 24	Thursday June 25
Control	184-186	187-189	190-192	193-195
Tritium				
1.5 Gy	205-206	207-208	209-210	211-212
3.0 Gy	219-220	221-222	223-224	225-226
4.5 Gy	233[a]-234	235-236	237-238	239-240
X-Rays				
1.5 Gy	247-248	249-250	251-252	253-254
3.0 Gy	261-262	263-264	265-266	267-268
4.5 Gy	275[a]-276	277-278	279-280	281-282

Treatment Group	Friday June 26	Tuesday June 30	Wednesday July 1
Control	196-198	199-201	202-204
Tritium			
1.5 Gy	213-214	215-216	217-218
3.0 Gy	227-228	229-230	231-232
4.5 Gy	241-242	243-244	245-246
X-Rays			
1.5 Gy	255-256	257-258	259-260
3.0 Gy	269-270	271-272	273-274
4.5 Gy	283-284	285-286	287[b]-288

a: Harvested on June 16, 1987 as a trial
b: Harvested on June 15, 1987 as a trial

APPENDIX III

Slide Evaluation - Phase I - Lymphocytes

Dose	Slide #	Quality	Comments
Control	1	Good	Very few metaphases
	2	Good	Very few metaphases
	3	Good	Very few metaphases
	4	Poor	Negligible metaphases
	5	OK	Very, very few metaphases
	6	Good	Few metaphases
	7	OK	Very few metaphases
	8	OK	Very few metaphases
	9	OK	Very, very few metaphases
	10	Good	Very few metaphases
	11	Good	Very few metaphases
	12	Good	Very few metaphases
	13	OK	Very few metaphases
	14	OK	Very few metaphases
	15	OK	Very few metaphases
Tritium	16	OK	10-15/slide
2.0 Gy	17	OK	10-15/slide
	18		
	19		
	20		
	21	Poor	Negligible
	22		
	23	Poor	Few cells, negligible metaphases
Tritium	24		
6.0 Gy	25		
	26	NA	No metaphases, No cells
	27		
	28		
	29	OK-poor	Very few metaphases
	30		
	31	OK	Negligible metaphases
X-rays	32	Good	Few metaphases - but enough
2.0 Gy	33	Poor	Few metaphases
	34		
	35		
	36		
	37		
	38		
	39	OK	Negligible metaphases

Dose	Slide #	Quality	Comments
X-rays	40		
6.0 Gy	41	OK	20/slide
	42		
	43		
	44		
	45		
	46		
	47		

Slide Evaluation - Phase I - Spleen Cells

Dose	Slide #	Quality	Comments
Control	1	Good	Centromeric staining
	2	OK	Centromeric staining/a bit pale
	3	Good	Centromeric staining
	4	Good	Centromeric staining
	5	Good	Centromeric staining
	6	Poor	Few metaphases
	7	Poor	Few metaphases
	8	Poor	Few metaphases
	9	Poor	Few metaphases
	10	Poor	Few metaphases
	11	Good	Overstained
	12	Good	Darkly stained
	13	OK	Fewer metaphases, dark staining
	14	Good	Very dark, fewer metaphases but enough
	15	Good	Very dark, fewer metaphases but enough
Tritium 2.0 Gy	16	Good	Centromeric staining
	17	Good	Centromeric staining
	18	Poor	Few metaphases, sister chromatids close together
	19	Poor	Few metaphases, sister chromatids close together
	20	Poor	Few metaphases, sister chromatids close together
	21	OK	Overstained, fewer metaphases
	22	OK	Fewer metaphases
	23	OK	Darkly stained
Tritium 6.0 Gy	24		No slide, mouse found dead
	25		No slide, mouse found dead
	26	OK	Few first metaphases
	27	OK	Few metaphases
	28	OK	OK
	29	Poor	Hardly any metaphases
	30	OK	Darkly stained, fewer metaphases
	31	OK	Darkly stained, fewer metaphases
X-rays 2.0 Gy	32	Good	Centromeric staining
	33	Good	Centromeric staining
	34	Good	Centromeric staining
	35	Poor	Very few metaphases, chromatids close together

Dose	Slide #	Quality	Comments
X-rays 2.0 Gy	36	Poor	Very few metaphases, chromatids close together
	37	OK	Overstained, fewer metaphases
	38	OK	Overstained, fewer metaphases
	39	OK	Overstained, fewer metaphases
X-rays 6.0 Gy	40	Good	Centromeric staining
	41	Good	Centromeric staining
	42	OK	Centromeric staining
	43	Poor	Very few metaphases, chromatids close together
	44	Poor	Very few metaphases, chromatids close together
	45	Poor	Very few metaphases, chromatids close together
	46	Good	Some centromeric staining
	47	Good	Darkly stained

Slides to be Scored - Phase I PBL

Control	1, 2, 3, 4, 5, 11, 12, 13, 14, 15
Tritium	
2.0 Gy	16, 17, 21, 22, 23
6.0 Gy	26, 27, 28, 30, 31
X-rays	
2.0 Gy	32, 33, 34, 37, 39
6.0 Gy	40, 41, 42, 46, 47

Slides to be Scored - Phase I Spleen Cells

Control	1, 2, 3, 4, 5, 11, 12, 13, 14, 15
Tritium	
2.0 Gy	16, 17, 21, 22, 23
6.0 Gy	26, 27, 28, 30, 31
X-rays	
2.0 Gy	32, 33, 34, 37, 39
6.0 Gy	40, 41, 42, 46, 47

Slide Evaluation - Phase II - PBL

Dose	Slide #	Quality	Comments
Control	48	Poor	Very few metaphases - tight spread
	49	Poor	Very few metaphases - tight spread
	50	Poor	Very few metaphases - tight spread
	51	Good	Nice spreads - many late divisions
	52	Good	
	53	OK	Low mitotic index
	54	Good	Many late divisions
	55	Good	Many late divisions
	56	Very good	High number of first divisions
	57	OK	Low mitotic index
	58	Good	
	59	Fair	Very low number of first divisions
	60	Very good	High mitotic index
	61	Good	
	62	OK	Low mitotic index
	63	Good	
	64	Good	
	65	OK	Low number of first division metaphases
	66	Good	
	67	Good	
	68	Good	
Tritium 1.5 Gy	69	Poor	
	70	Poor	
	71	Poor	
	72	Poor	
	73	Fair	Very low mitotic index
	74	Fair	Low mitotic index - poor spreads
	75	Good	Many late division metaphases
	76	Fair	Low mitotic index
	77	Fair	Low mitotic index
	78	Fair	Low mitotic index
	79	Fair	Low mitotic index
	80	Fair	Low mitotic index
	81	Fair	Low mitotic index
	82	Fair	Low mitotic index
	83	Fair	Low mitotic index
	84	Fair	Low mitotic index
	85	Fair	Very low mitotic index
	86	Good	
	87	Good	
	88	Good	
	89	OK	Not many metaphases
	90	Good	

Dose	Slide #	Quality	Comments
Tritium	91	Good	
1.5 Gy	92	OK	Few metaphases
	93		Very good
	94	Very good	
	95	Very good	
Tritium	96	Poor	
3.0 Gy	97	Poor	
	98	Poor	
	99	Fair	Very low mitotic index
	100	Below average	Extremely low mitotic index - few nice spreads
	101	OK	
	102	OK	
	103	Poor	
	104	OK	
	105	Poor	Very low mitotic index
	106	Poor	Very low mitotic index
	107	Poor	Very low mitotic index
	108	Very good	Many metaphases
	109	Good	
Tritium	110	Poor	
4.5 Gy	111	Poor	
	112	OK	
	113	OK	Spreads tend to be tight
	114	Good	
	115	Good	
	116	Below average	Spreads are good, but very few of them
	117	OK	
	118	OK	
	119	Fair	Low mitotic index
	120	Fair	Low mitotic index
	121	Fair	Low mitotic index
	122	Fair	Low mitotic index
	123	Good	
X-rays	124	Poor	
1.5 Gy	125	Poor	
	126	Poor	
	127	Poor	
	128	Good	
	129	OK	
	130	Very good	
	131	Very good	

Dose	Slide #	Quality	Comments
X-rays 1.5 Gy	132	Fair	Few metaphases - some are well spread
	133	Fair	
	134	Poor	
	135	Good	
	136	Good	
	137	Good	
	138	OK	
	139	Fair	Low mitotic index
	140	OK	
	141	Good	
	142	Very good	
	143	Fair	
	144	Very good	Lots of metaphases
	145	Very good	
	146	Good	
	147	Good	
	148	OK	
	149	OK	
	150	Fair	
	151	Good	
	152	Good	
	153	Poor	
X-rays 3.0 Gy	154	Poor	
	155	Poor	
	156	Fair	
	157	Fair	
	158	Fair	
	159	OK	
	160	Fair	
	161	Fair	
	162	Below average	Very, very few metaphases
	163	OK	
	164	Fair	
	165	Fair	
	166	Very good	This may have been mixed with #153 (1.5 Gy) and/or #167 (3.0 Gy)
	167	Good	
	168	Very good	
X-rays 4.5 Gy	169	Poor	
	170	Poor	
	171	Poor	
	172	Fair	

Dose	Slide #	Quality	Comments
X-rays	173	OK	
4.5 Gy	174	Poor	
	175	Fair	
	176	Below average	Very few metaphases - well spread
	177	OK	
	178	Good	
	179	OK	
	180	OK	
	181	Good	
	182	Very good	
	183	Very good	

Slide Evaluation - Phase II - Spleen Cells

Dose	Slide #	Quality	Comments
Control	48	Fair	Some tight metaphases
	49	Fair	Some tight metaphases
	50	OK	Some tight metaphases
	51	Very poor	Tight metaphases - lots of cytoplasmic background
	52	Very poor	Tight metaphases - lots of cytoplasmic background
	53	Poor	Tight metaphases (some scorable ones)
	54	Very good	Second slide, lighter stained
	55	Good	Nice spreads
	56	Good	Nice spreads
	57	Good	
	58	Good	
	59	Good	
	60	Good	
	61	Good	
	62	Good	
	63	Good	
	64	Good	
	65	Good	
	66	Good	
	67	Good	
	68	Good	
Tritium 1.5 Gy	69	Very poor	Tight metaphases - faint staining
	70	Very poor	Tight metaphases - faint staining
	71	Poor	Few scorable metaphases - mostly tight
	72	Fair	More scorable metaphases
	73	Poor	Faint staining
	74	Poor	Faint staining - some scorable metaphases
	75	Poor	
	76	Very poor	
	77	OK	Many broken metaphases - nice spreads
	78	Good	
	79	Good	
	80	Good	
	81	Good	
	82	Very good	
	83	Good	
	84	Good	
	85	Good	

Dose	Slide #	Quality	Comments
Tritium	86	Good	
1.5 Gy	87	Good	
	88	Good	
	89	Very good	
	90	Good	
	91	Good	
	92	Good	
	93	Good	
	94	Good	
	95	Good	
Tritium	96	Fair	Many scorable metaphases -
3.0 Gy			many tight metaphases
	97	Poor	Tight metaphases
	98	Very poor	
	99	Poor	
	100	Good	
	101	Good	
	102	Good	
	103	Good	
	104	Good	
	105	Good	Slightly overcrowded
	106	Good	
	107	Good	
	108	Good	
	109	Good	
Tritium	110	Fair	Some tight metaphases -
4.5 Gy			many scorable
	111	Poor	Few scorable metaphases
	112	Poor	Few scorable metaphases
	113	Very poor	Tight metaphases
	114	Good	
	115	Good	
	116	Good	
	117	Good	
	118	Good	
	119	Good	
	120	Good	
	121	Good	
	122	Very good	
	123	Good	
X-rays	124	Poor	Few scorable metaphases -
1.5 Gy			mostly tight
	125	Fair	Many scorable metaphases
	126	Poor	Tight metaphases

Dose	Slide #	Quality	Comments
X-rays	127	Poor	
1.5 Gy	128	Poor	
	129	Poor	
	130	Poor	
	131	Very poor	
	132	OK	Some broken metaphases
	133	OK	Many broken metaphases - well spread though
	134	OK	Many broken metaphases
	135	Good	
	136	Good	
	137	Good	
	138	Good	
	139	Good	
	140	Good	
	141	Good	
	142	Good	
	143	Good	
	144	Good	
	145	Good	
	146	Good	
	147	Good	
	148	Good	
	149	Good	
	150	Good	
	151	Good	
	152	Good	
	153	Good	
X-rays	154	Poor	Some scorable metaphases
3.0 Gy	155	Fair	
	156	Poor	
	157	Poor	
	158	Good	
	159	Good	
	160	Good	
	161	Good	
	162	Good	
	163	Good	Slightly overstained
	164	Very good	
	165	Good	
	166	Good	
	167	Very Poor	Very tight metaphases - few scorable ones
	168	Good	

Dose	Slide #	Quality	Comments
X-rays	169	Fair	Metaphases not great but scorable
3.0 Gy	170	Fair	Metaphases not great but scorable
	171	Poor	Few scorable metaphases
	172	Very poor	Tight metaphases
X-rays	173	Good	
4.5 Gy	174	Good	
	175	Good	
	176	Good	
	177	Good	
	178	Good	
	179	Good	
	180	Good	
	181	Good	
	182	Good	
	183	Good	

Slides to be Scored - Phase II - PBL

Control	51, 52, 53, 54, 55, 56, 60, 61, 63, 64
Tritium	
1.5 Gy	73, 75, 76, 77, 78, 79, 80, 81, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95
3.0 Gy	96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109
4.5 Gy	114, 115, 116, 117, 118, 119, 120, 121, 122, 123
X-rays	
1.5 Gy	128, 129, 130, 131, 135, 136, 137, 138, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 151, 152
3.0 Gy	156, 157, 158, 159, 160, 163, 164, 165, 167, 168
4.5 Gy	172, 173, 175, 177, 178, 179, 180, 181, 182, 183

Slide Evaluation - Spermatocytes

Dose	Slide #	Quality	Comments
Control	184	Good	
	185	Good	
	186	OK	Many spreads are tight
	187	OK	
	188	Good	
	189	Good	
	190	Good	
	191	OK	Lightly stained metaphases, but nice-looking
	192	Poor	Few cells, few, poor-looking meioses
	193	OK	
	194	OK	
	195	OK	
	196	Good	
	197	Fair	Chromosomes somewhat fuzzy
	198	Fair	Some metaphases have fuzzy chromosomes
	199	Good	Few cells and metaphases, but nice-looking
	200	Good	Lots of sperm cells on slides
	201	OK	
	202	OK	
	203	Good	Good quality spreads
	204	Good	
Tritium 1.5 Gy	205	Good	
	206	OK	Spreads are somewhat tight
	207	Good	
	208	OK	
	209	Fair	Not a lot of metaphases
	210	Poor	Few cells, no metaphases observed
	211	Good	Lots of sperm cells in some areas of slide
	212	Poor	Poor chromosome coiling, no nice metaphases observed
	213	OK	
	214	Poor	Tight spreads, poor chromosome coiling

Dose	Slide #	Quality	Comments
Tritium	215	Good	
1.5 Gy	216	OK	
	217	Good	
	218	Fair	Fuzzy chromosomes
Tritium	219	Good	
3.0 Gy	220	Poor	Few cells, no metaphases observed
	221	Fair	Chromosomes somewhat fuzzy
	222	Fair	
	223	Poor	Very few cells, no meioses observed
	224	Poor	Very few cells, no meioses observed
	225	Fair	Few spreads, not good quality
	226	Poor	Preparations are not too nice, some spreads are OK
	227	Good	
	228	Fair	Fuzzy chromosomes
	229	Poor	Very few cells, some meioses are OK
	230	OK	Metaphases somewhat scarce
	231	Fair	Few metaphases, somewhat tight
	232	OK	Few meiotic spreads, but nice-looking
	233	Fair	Few cells, meioses are fair
	234	Poor	Very few cells, no meioses observed
	235	Poor	No meioses observed, very few cells on slides
	236	Poor	Very few cells, no metaphases observed
	237	Poor	Very few cells, no metaphases observed
	238	Poor	Very few cells, no metaphases observed
	239	Poor	Very few cells, no meioses observed
	240	Poor	Few cells, very few metaphases observed, fair

Dose	Slide #	Quality	Comments
Tritium 4.5 Gy	241	Poor	Hardly any cells, one meiotic spread observed, OK
	242	Poor	Few cells, one metaphase observed, OK
	243	OK	Very few cells, meioses (few of them) are OK
	244	Poor	Few cells, some metaphases observed, fair
	245	Poor	Very few cells, couple of meioses observed were good
	246	Poor	Few cells, couple of metaphases observed, OK
X-rays 1.5 Gy	247	Good	
	248	Fair	Meiotic spreads are rather tight
	249	Fair	Metaphases have fuzzy chromosomes
	250	Good	
	251	Fair	Fuzzy chromosomes
	252	Fair	Chromosomes tend to be fuzzy
	253	Good	
	254	OK	Rather few cells, but plenty of metaphases
	255	Good	
	256	Fair	Many sperm cells in preps - fuzzy chromosomes
	257	Good	
	258	OK	Many sperm cells in preparations
	259	Good	
	260	Fair	
X-rays 3.0 Gy	261	Fair	Few cells on slides, the few metaphases observed were OK
	262	OK	
	263	Good	Fewer cells than usual, nice spreads
	264	Good	
	265	OK	
	266	OK	Rather few cells
	267	OK	
	268	OK	Fuzzy chromosomes
	269	OK	

Dose	Slide #	Quality	Comments
X-rays	270	OK	
3.0 Gy	271	Poor	Few metaphases, fuzzy chromosomes
	272	OK	
	273	Poor	Very light staining, faintly stained chromosomes
	274	Fair	Low mitotic index, fuzzy chromosomes
X-rays	275	Good	
4.5 Gy	276	Good	
	277	Poor	Few cells, some good metaphases observed,
	278	Poor	Very few cells, meioses observed were OK
	279	Poor	Very poor preparations, cells look like they are full of holes
	280	Poor	Very few cells, no metaphases observed
	281	Good	Good quality meiotic spreads but very few cells
	282	OK	Few metaphases, nice spreads
	283	Fair	Few cells on slides, meioses OK
	284	Poor	Very few cells, couple of meioses
	285	OK	Chromosomes slightly fuzzy
	286	Fair	Very fuzzy chromosomes
	287	Fair	Few meioses, nice looking chromosomes
	288	Poor	Very few cells, the few meioses that are present are OK

Slides to be Scored - Spermatocytes

Control	184, 185, 187, 188, 189, 190, 191, 193, 194, 196, 199, 200, 202, 203, 204
Tritium	
1.5 Gy	205, 206, 207, 208, 211, 213, 215, 216, 217, 218
3.0 Gy	219, 221, 222, 225, 227, 228, 229, 230, 231, 232
4.5 Gy	233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246
X-rays	
1.5 Gy	247, 250, 253, 254, 255, 256, 257, 259, 260
3.0 Gy	261, 262, 263, 264, 265, 266, 267, 269, 270, 272
4.5 Gy	275, 276, 277, 278, 281, 282, 283, 285, 286, 287.

APPENDIX IV

PROJECT NO.: 00089

PHASE II - PBL SLIDE CODING

Treatment Group	Animal Number	Code	Treatment Group	Animal Number	Code
Control	51	21		84	55
	52	6		85	72
	53	11		86	64
	54	19		87	5
	55	60		88	39
	56	84		89	66
	60	14		90	85
	61	37		91	77
	63	82		92	79
	64	87		93	1
				94	40
	73	91			
Tritium 1.5 Gy	75	59		95	74
	76	30		96	75
				97	94
				98	92
	77	47	Tritium 3.0 Gy	99	32
	78	10		100	17
	79	20		101	69
	80	48		102	52
				103	93
	81	2		104	62
	83	51		105	90

Performed by: Sharon Tell Date: 1/6/87

Reviewed by: [signature] Date: June 1/87

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PROJECT NO.: 00089

PHASE II - PBL SLIDE CODING

Treatment Group	Animal Number	Code	Treatment Group	Animal Number	Code
	106	45		131	86
	107	22		135	23
	108	65		136	41
	109	50		137	81
				138	36
Tritium 4.5 Gy	114	71		140	29
	115	44		141	46
	116	68		142	89
	117	31		143	61
	118	27		144	25
	119	3		145	13
	120	75		146	88
	121	80		147	8
	122	76		148	53
	123	18		149	16
				151	7
X-rays 1.5 Gy	128	67		152	42
	129	35			
	130	9			

Performed by: Shara Tol Date: 1/6/87

Reviewed by: [signature] Date: June 1/87

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PROJECT NO.: [redacted]

PHASE II - PBL SLIDE CODING

Treatment Group	Animal Number	Code	Treatment Group	Animal Number	Code
X-ray 3.0 Gy	156	26		182	49
	157	34		183	70
	158	12			
	159	43			
	160	28			
	163	4			
	164	78			
	165	57			
	167	33			
	168	73			
X-ray 4.5 Gy	172	93			
	173	38			
	175	56			
	177	15			
	178	54			
	179	24			
	180	58			
	181	63			

Performed by: Sharon Tod Date: 1/6/87

Reviewed by: [signature] Date: June 1/87

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PROJECT NO.: 00089

SPERMATOCYTE SLIDE CODING

Treatment Group	Animal Number	Code	Treatment Group	Animal Number	Code
CONTROL	184	111	Tritium	208	116
	185	134	1.5 Gy	211	160
	187	147	(Continued)	213	105
	188	102		215	129
	189	128		216	154
	190	142		217	124
	191	153		218	167
	193	115			
	194	159	Tritium	219	136
	196	166	3.0 Gy	221	170
	199	123		222	110
	200	172		225	173
	202	162		227	141
	203	109		228	161
	204	135		229	130
				230	149
Tritium	205	143		231	125
1.5 Gy	206	126		232	104
	207	117			

Performed by: C Behar Date: Aug 31/87

Reviewed by: [signature] Date: NOV 18 1987

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PROJECT NO.: 00059

SPERMATOCYTE SLIDE CODING

Treatment Group	Animal Number	Code	Treatment Group	Animal Number	Code
Tritium	235	133			
	236	138	X-ray	254	112
4.5 Gy	237	176	1.5 Gy (cont'd)	260	148
	238	189			
	239	145			
	240	119	X-ray	261	121
	241	150	3.0 Gy	262	151
	242	156		263	158
	243	108		264	175
	244	164		265	132
	245	171		266	169
	246	139		267	144
				269	106
X-ray	247	168		270	163
1.5 Gy	250	174		272	155
	253	118			
	254	165	X-ray	275	152
	255	157	4.5 Gy	276	133
	256	131		277	122
	257	107		278	140
	258	146		281	113

Performed by: D Bekar Date: Aug 31/87

Reviewed by: [signature] Date: Nov 18, 1987

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PROJECT NO.: 00089

SPERMATOCYTE SLIDE CODING

Treatment Group	Animal Number	Code	Treatment Group	Animal Number	Code
X-ray	282	103			
4.5 Gy	283	120			
(continued)	285	127			
	286	137			
	287	114			

Performed by: DBehas Date: Aug 31/87

Reviewed by: [signature] Date: NOV 15 1987

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APPENDIX V

Blood Volume Recuperation and Culture Record - Phase I

Dose Group	Animal Code	Blood Withdrawn (ml)	No. of Cultures
Control	1	0.4	2
	2	0.5	3
	3	0.6	3
	4	0.2	1
	5	0.7	4
	6	0.8	4
	7	0.8	4
	8	0.5	3
	9	0.6	3
	10	0.6	3
	11	0.7	4
	12	0.6	3
	13	0.6	3
	14	0.6	3
	15	0.6	3
Tritium 2.0 Gy	16	0.5	3
	17	0.9	5
	18	0.7	4
	19	0.5	3
	20	0.8	4
	21	0.7	4
	22	0.5	3
	23	0.8	4
Tritium 6.0 Gy	24	N/A	N/A) found dead on
	25	N/A	N/A) Feb. 17/87
	26	0.7	4
	27	0.5	3
	28	0.7	4
	29	0.8	4
	30	0.0	0
	31	0.7	4
X-rays 2.0 Gy	32	0.9	5
	33	0.8	4
	34	0.8	4
	35	0.8	4
	36	0.8	4
	37	0.8	4
	38	0.6	3
	39	0.6	3

Dose Group	Animal Code	Blood Withdrawn (ml)	No. of Cultures
X-rays	40	0.8	4
6.0 Gy	41	0.8	4
	42	0.7	4
	43	0.8	4
	44	0.7	4
	45	0.8	4
	46	0.8	4
	47	0.8	4

Blood Volume Recuperation and Culture Record - Phase II

Dose Group	Prim. Animal Code	Sec. Slide Code	Blood Withdrawn (ml)	No. of Cultures
Control	48		0.80	4
	49		0.60	3
	50		0.40	2
	51	21	0.70	4
	52	6	0.70	4
	53	11	0.75	4
	54	19	0.75	4
	55	60	0.70	4
	56	84	0.70	4
	57		0.65	3
	58		0.75	4
	59		0.70	4
	60	14	0.55	3
	61	37	0.55	3
	62		0.65	3
	63	82	0.60	3
	64	87	0.65	3
	65		0.70	3
	66		0.70	3
	67		0.55	3
	68		0.60	3
Tritium 1.5 Gy	69		0.90	4
	70		0.90	4
	71		0.60	3
	72		0.60	3
	73	91	0.75	4
	74		0.60	3
	75	59	0.60	3
	76	30	0.80	4
	77	47	0.65	3
	78	10	0.60	3
	79	20	0.85	4
	80	48	0.70	3
	81	2	0.65	3
	82		0.65	3
	83	51	0.45	2
	84	55	0.65	3
	85	72	0.50	3
	86	64	0.65	3
	87	5	0.45	2
	88	39	0.40	2

Dose Group	Prim. Animal Code	Sec. Slide Code	Blood Withdrawn (ml)	No. of Cultures
Tritium	89	66	0.80	4
1.5 Gy	90	85	0.65	3
	91	77	0.45	2
	92	79	0.45	2
	93	1	0.60	3
	94	40	0.55	3
	95	74	0.65	3
Tritium	96	95	0.40	2
3.0 Gy	97	94	0.50	3
	98	92	0.40	2
	99	32	0.40	2
	100	17	0.65	3
	101	69	0.40	2
	102	52	0.40	2
	103	93	0.60	3
	104	62	0.40	2
	105	90	0.45	2
	106	45	0.50	3
	107	22	0.55	3
	108	65	0.50	3
	109	50	0.60	3
Tritium	110		0.10	1
4.5 Gy	111		0.60	3
	112		0.80	4
	113		0.60	3
	114	71	0.75	4
	115	44	0.80	4
	116	68	0.60	3
	117	31	0.60	3
	118	27	0.70	4
	119	3	0.80	4
	120	75	0.40	2
	121	80	0.55	3
	122	76	0.60	3
	123	18	0.60	3
X-rays	124		0.80	4
1.5 Gy	125		0.60	3
	126		0.40	2
	127		0.60	3
	128	67	0.60	3
	129	35	0.80	4

Dose Group	Prim. Animal Code	Sec. Slide Code	Blood Withdrawn (ml)	No. of Cultures
X-rays	130	9	0.85	4
1.5 Gy	131	86	0.60	3
	132		0.50	3
	133		0.80	4
	134		0.25	1
	135	23	0.55	3
	136	41	0.80	4
	137	81	0.80	4
	138	36	0.75	4
	139		0.60	3
	140	29	0.70	4
	141	46	0.60	3
	142	89	0.60	3
	143	61	0.65	3
	144	25	0.80	4
	145	13	0.50	3
	146	88	0.60	3
	147	8	0.60	3
	148	59	0.50	3
	149	16	0.20	1
	150		0.65	3
	151	7	0.55	3
	152	42	0.65	3
	153		0.85	4
X-rays	154		0.80	4
3.0 Gy	155		0.60	3
	156	26	0.90	4
	157	34	0.80	4
	158	12	1.00	4
	159	43	0.90	4
	160	28	0.85	4
	161		0.50	3
	162		0.65	3
	163	4	0.55	3
	164	78	0.70	3
	165	57	0.45	2
	166		0.55	3
	167	33	0.55	3
	168	73	0.55	3

Dose Group	Prim. Animal Code	Sec. Slide Code	Blood Withdrawn (ml)	No. of Cultures
X-rays 4.5 Gy	169		0.80	4
	170		0.80	4
	171		0.40	2
	172	83	0.50	3
	173	38	0.60	3
	174		0.30	2
	175	56	0.60	3
	176		0.50	3
	177	15	0.65	3
	178	54	0.50	3
	179	24	0.80	4
	180	58	0.75	4
	181	63	0.80	4
	182	49	0.50	3
	183	70	0.60	3

APPENDIX VI

Spleen Cell Number Record - Phase I

Dose Group	Animal Code	Total # of Spleen Cells ($\times 10^7$)	Means $\pm$ S.D. ($\times 10^7$)
Control	1	8.80	
	2	13.52	
	3	8.88	
	4	8.48	
	5	7.68	
	6	11.60	
	7	10.16	
	8	12.08	
	9	12.80	
	10	5.80	
	11	7.28	
	12	6.24	
	13	7.12	
	14	15.04	
	15	6.64	9.47 $\pm$ 2.90
Tritium 2.0 Gy	16	4.56	
	17	5.68	
	18	4.20	
	19	4.40	
	20	5.52	
	21	6.24	
	22	3.52	
	23	5.12	4.91 $\pm$ 0.89
Tritium 6.0 Gy	24	N/A) found dead on	
	25	N/A) Feb. 17/87	
	26	0.32	
	27	0.25	
	28	29.60	
	29	29.20	
	30	10.00	
	31	23.20	15.43 $\pm$ 13.70
X-rays 2.0 Gy	32	6.92	
	33	10.80	
	34	10.00	
	35	4.44	
	36	4.64	
	37	9.04	
	38	8.56	
	39	6.56	7.62 $\pm$ 2.37

Dose Group	Animal Code	Total # of Spleen Cells ($\times 10^7$)	Means $\pm$ S.D. ($\times 10^7$)
X-rays 6.0 Gy	40	4.48	
	41	5.28	
	42	5.92	
	43	3.04	
	44	4.16	
	45	3.12	
	46	2.64	
	47	4.08	4.09 ± 1.14

Spleen Cell Number Record - Phase II

Dose Group	Prim. Animal Code	Sec. Slide Code	Total # of Spleen Cells ($\times 10^7$)	Means $\pm$ S.D. ($\times 10^7$)
Control	48		11.06	
	49		9.30	
	50		9.40	
	51	21	10.64	
	52	6	10.40	
	53	11	8.48	
	54	19	14.64	
	55	60	12.64	
	56	84	5.76	
	57		11.36	
	58		9.04	
	59		9.92	
	60	14	5.68	
	61	37	13.90	
	62		9.52	
	63	82	9.84	
	64	87	7.44	
	65		10.88	
	66		9.10	
	67		13.60	
	68		6.90	9.98 $\pm$ 2.44
Tritium 1.5 Gy	69		6.80	
	70		6.70	
	71		5.40	
	72		6.40	
	73	91	6.48	
	74		5.36	
	75	59	6.88	
	76	30	6.44	
	77	47	4.80	
	78	10	4.88	
	79	20	6.96	
	80	48	8.80	
	81	2	5.92	
	82		7.12	
	83	51	5.76	
	84	55	5.00	
	85	72	5.36	
	86	64	7.76	
	87	5	6.00	
	88	39	7.20	

Dose Group	Prim. Animal Code	Sec. Slide Code	Total # of Spleen Cells ($\times 10^7$)	Means $\pm$ S.D. ($\times 10^7$)
Tritium	89	66	4.60	
1.5 Gy	90	85	5.68	
	91	77	4.88	
	92	79	4.10	
	93	1	9.40	
	94	40	7.70	
	95	74	6.00	6.24 $\pm$ 1.27
Tritium	96	95	5.30	
3.0 Gy	97	94	3.96	
	98	92	3.44	
	99	32	4.48	
	100	17	2.46	
	101	69	4.08	
	102	52	3.80	
	103		2.92	
	104	64	3.68	
	105	90	4.96	
	106	45	4.00	
	107	22	4.42	
	108	65	4.20	
	109	50	3.20	3.92 $\pm$ 0.77
Tritium	110		3.46	
4.5 Gy	111		6.64	
	112		4.44	
	113		5.76	
	114	71	3.52	
	115	44	3.48	
	116	68	3.16	
	117	31	3.56	
	118	27	4.16	
	119	3	4.00	
	120	75	4.60	
	121	80	2.88	
	122	76	3.30	
	123	18	3.80	4.05 $\pm$ 1.04
X-rays	124		7.10	
1.5 Gy	125		7.60	
	126		6.10	
	127		6.30	
	128	67	8.32	
	129	35	4.64	

Dose Group	Prim. Animal Code	Sec. Slide Code	Total # of Spleen Cells $(\times 10^7)$	Means $\pm$ S.D. $(\times 10^7)$
X-rays	130	9	4.40	
1.5 Gy	131	86	4.88	
	132		6.32	
	133		8.40	
	134		8.48	
	135	23	7.20	
	136	41	6.48	
	137	81	4.28	
	138	36	4.48	
	139		8.00	
	140	29	7.04	
	141	46	5.76	
	142	89	6.96	
	143	61	6.96	
	144	25	7.40	
	145	13	6.80	
	146	88	6.80	
	147	8	7.30	
	148	53	5.50	
	149	16	7.50	
	150		5.76	
	151	7	5.12	
	152	42	7.12	
	153		7.28	6.54 ± 1.22
X-rays	154		4.80	
3.0 Gy	155		6.04	
	156	26	4.96	
	157	34	5.68	
	158	12	5.64	
	159	43	8.24	
	160	28	4.80	
	161		4.04	
	162		6.08	
	163	4	5.68	
	164	78	6.10	
	165	57	5.66	
	166		7.36	
	167	33	5.44	
	168	73	6.08	5.77 ± 1.02

Dose Group	Prim. Animal Code	Sec. Slide Code	Total # of Spleen Cells ($\times 10^7$)	Means $\pm$ S.D. ($\times 10^7$)
X-rays	169		5.40	
4.5 Gy	170		4.02	
	171		5.52	
	172	83	4.08	
	173	38	4.52	
	174		4.84	
	175	56	5.20	
	176		6.80	
	177	15	4.36	
	178	54	4.32	
	179	24	4.68	
	180	58	3.80	
	181	63	5.20	
	182	49	3.48	
	183	70	4.36	4.71 ± 0.83

APPENDIX VII

Testicular Weight Record

Dose Group	Prim. Animal Code	Sec. Slide Code	Weight of 2 Testes (mg)	Mean ± S.D.
Control	195		110.0	
	196	166	131.2	
	197		106.3	
	198		138.5	
	199	123	114.0	
	200	172	161.1	
	201		132.1	
	202	162	122.1	
	203	109	123.2	
	204	135	125.1	126.4 ± 15.86
Tritium	212		100.8	
1.5 Gy	213	105	104.0	
	214		119.8	
	215	129	106.3	
	216	154	96.8	
	217	124	107.5	
	218	167	112.8	106.9 ± 7.63
Tritium	226		69.8	
3.0 Gy	227	141	57.9	
	228	161	66.9	
	229	130	52.8	
	230	149	59.1	
	231	125	61.7	
	232	104	67.0	62.2 ± 6.05
Tritium	240	119	40.5	
4.5 Gy	241	150	38.3	
	242	156	48.4	
	243	108	46.4	
	244	164	42.9	
	245	171	33.0	
	246	139	37.5	41.0 ± 5.34
X-rays	255	157	118.9	
1.5 Gy	256	131	107.1	
	257	107	98.4	
	258	146	106.9	
	259	112	102.4	
	260	148	116.2	108.3 ± 7.89

Dose Group	Prim. Animal Code	Sec. Slide Code	Weight of 2 Testes (mg)	Mean ± S.D.
X-rays	269	106	68.4	
3.0 Gy	270	163	89.5	
	271		90.8	
	272	155	77.6	
	273		81.1	
	274		80.5	81.3 ± 8.23
X-rays	283	120	52.7	
4.5 Gy	284		55.7	
	285	127	50.2	
	286	137	58.6	
	287	114	45.9	52.6 ± 4.91

APPENDIX VIII

CHROMOSOMAL ABERRATION SCORE SHEET

This form is the standard Chromosomal Aberration Score Sheet used for the recording of chromosomal damage observed in peripheral blood and/or spleen lymphocytes of Phase I and Phase II stages of project 00089.

The slide number and experiment number were recorded on the top right hand corner of the sheet. The microscope number, the name of the individual scoring the slide, the date the slide was scored, and the orientation of the slide on the stage were recorded on the top of the sheet.

On each sheet, 50 cells may be scored. The "LOCATION" columns indicate the location of each metaphase on the "x" and "y" co-ordinates of the vernier scales of the microscope.

Normal metaphases are recorded in the "NOR" column. Cells with chromosomal damage are recorded either under the "CHROMOSOME" or "CHROMATID" sections of the score sheet depending on what type of damage was observed.

"CHROMOSOME" type damage included: dicentrics (DIC), centric or acentric rings (RING), or deletions (DEL). Damages such as translocations, inversions, chromosome gaps, etc. were included under the "OTHER" column.

"CHROMATID" type damage included: interchange (INTER), intrachange (INTRA), triradials (TRI), isochromatid deletions (ISO), and chromatid deletions (DEL). Chromatid gaps were included in the "OTHER" column.

If a cell had more than one chromosomal aberration, the extra aberrations were noted under the "EXTRA" columns.

Where required, notes were written under the "NOTE" column. If insufficient space was available, the back of the score sheet was used. The totals for each column were given at the bottom of each score sheet in the "TOTALS" row.

All score sheets were signed and dated.

CHROMOSOMAL ABERRATION SCORE SHEET

SLIDE NO. _____

EXPT. NO. _____

SCORED ON MICROSCOPE NO. _____ BY _____ ON _____ (DATE)

WITH THE LABEL TO THE LEFT/RIGHT.

	LOCATION		NOR	CHROMOSOME				CHROMATID						* EXTRA NOTES				
	X	Y		DIC	RING	DEL	OTHER	INTER	INTRA	TRI	ISO	DEL	OTHER					
1																		
2																		
3																		
4																		
5																		
6																		
7																		
8																		
9																		
10																		
11																		
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48																		
49																		
50																		
TOTALS																		

SCORED BY (SIGNATURE, DATE)

CHECKED BY (SIGNATURE, DATE)

APPENDIX IX

RECIPROCAL TRANSLOCATION SCORE SHEET

On the top of the score sheet, the following information was recorded: Slide number, experiment number, microscope number, scorer's name, date the slide was scored, and slide orientation on the microscope stage. The x and y co-ordinates or the vernier scales of the microscope of each of the 50 metaphases recorded on each score sheet were recorded under the "LOCATION" column. Normal metaphases were recorded under the "NOR" column.

Translocations were recorded either under the "RINGS" or "CHAINS" sections depending on the type observed. The "RING" columns were labelled as follows:

RIV = ring of four
RVI = ring of six
RVIII = ring of eight
RX = ring of ten

The "CHAIN" columns were labelled as follows:

CII+II = chain of two and two, resulting from a translocation in which two cross-overs have occurred both proximal or both distal to the translocation.
CIII+I = a chain of three + one univalent
CIV = chain of four
CVI = chain of six
CVIII = chain of eight
CX = chain of ten

Univalents, deletions and gaps observed were grouped together under the "OTHER" category. Extra aberrations were noted as "EXTRA" in one of the three remaining blank columns.

All score sheets were signed and dated.

RECIPROCAL TRANSLOCATION SCORE SHEET

SLIDE NO. _____
EXPT. NO. _____

SCORED ON MICROSCOPE NO. _____ BY _____ ON _____ (DATE)
WITH THE LABEL TO THE LEFT/RIGHT.

	LOCATION		NOR	RINGS				CHAINS						OTHER				
	X	Y		R IV	R VI	R VIII	R X	C II+III	C III+I	C IV	C VI	C VIII	C X					
1																		
2																		
3																		
4																		
5																		
6																		
7																		
8																		
9																		
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47																		
48																		
49																		
50																		
TOTALS																		

SCORED BY (SIGNATURE, DATE)

CHECKED BY (SIGNATURE, DATE)

APPENDIX X

Radiation Quantities, Symbols, and Values Listed in Old Units and International System of Units (SI)

Quantity	Old Unit		SI Unit		
Name	Name	Symbol	Name	Symbol	Value
Activity	curie	Ci	becquerel	Bq	1 s^{-1}
					1 Bq = 2.7×10^{-11} Ci
					1 Ci = 3.7×10^{10} Bq
Exposure	roentgen	R	--		1 C/kg = 3876 R
					1 R = 2.58×10^{-4} C/kg
Absorbed Dose	rad	rad	gray	Gy	1 J/kg
					1 Gy = 100 rad
					1 rad = 0.01 Gy
Dose Equivalent	rem	rem	sievert	Sv	1 J/kg
					1 Sv = 100 rem
					1 rem = 0.01 Sv

APPENDIX XI

Physics & Health Sciences
Radiation Biology Branch

1987 February 12

WHITE NOTE

Animals received on February 13/87

TO: C. Chopra

FROM: N. Gragtmans

The shipment of CBA mice, Phase I, includes 7 crates of female mature animals.

Crate A - 15 control

Crate B - 8 animals received approximately 2 Gy X-rays during the period 87.02.02 - 12. Dose rates were adjusted to simulate those received upon injection of HTO (IP)
i.e. with biological halflife of 2 days

Crate C - 8 animals received, as for crate B, except at a nominal dose of 6 Gy

Crate D - 8 animals received 7.2 mCi HTO (IP) per mouse on 87.02.02. The nominal dose is 2 Gy

Crate E - 8 animals received 21.6 mCi HTO (IP) per mouse on 87.02.02. The nominal dose is 6 Gy

Crate F - 2 animals received HTO on 87.01.21 (IP) at a dose of approximately 6 Gy

Crate G - 5 animals received, as for Crate F, except injection was on 87.01.23

Accurate dosimetry for Crates B and C was done by TLD's and for Crates D and E by urine assay. J.R. Johnson will inform you on the calculated doses for these crates. Crates F and G represent pilot study groups, used to assess clinical effects of this protocol. Dosimetry for these last two crates is estimated slightly in excess of 6 Gy.

N.J. Gragtmans

NJG:lm

c: J.R. Johnson
D.K. Myers

Physics & Health Sciences
Radiation Biology Branch

1987 April 22

Animals received on April 23/87

CC

WHITE NOTE

TO: C. Chopra

FROM: N. Gragtmans

The shipment of CBA mice, Phase II, includes 8 crates of animals. Tritium groups were treated on 87 April 06. On this date X-irradiation was started for the X-ray groups for a total of 10 days, halving the doserate every second day. Dosimetry will be communicated to you by separate report from J.R. Johnson. Following is the shipment inventory. Note that each of seven animals in each tritium cage has been ear-tagged 1 through 7 for individual dose assignment. I included a copy of the ear-tag code for your information. Should you have any questions, please feel free to contact me.

CRATE	GROUP	CAGE	SEX	DOB	ANIMAL	WEIGHT (gr) AT TREATMENT
1	Control	Control 1	F	-		
		Control 2	M	87.01.20-26		
2	X-ray 1.5	A-2	7 M	87.01.20		
		A-3	8 F	-		
		A-4	7 F	-		
		A-5	7 M	87.01.14-15		
3	X-ray 1.5	A-9	7 F	-		
		A-10	8 F	-		
	Tritium 1.5	HTO-A	M	87.01.15	1	20.4
					2 — Tag torn.	23.7
					3	22.1
					4 - ½ hole high on ear.	21.3
					5	24.0
					6	24.3
					7	21.3
		HTO-B	M	87.01.14	1	20.8
					2	22.4
					3	22.2
					4	20.6
					5	20.9
					6	18.4
					7	19.6

CRATE	GROUP	CAGE	SEX	DOB	ANIMAL	WEIGHT (gr) AT TREATMENT	
4	X-ray 3.0	B-8	7 M	87.01.18-19			
		B-9	8 F				
		B-10	7 M	87.01.02-14			
		B-11	7 F				
5	X-ray 4.5	C-2	7 M	87.01.15			
		C-3	8 F				
		C-4	7 M	87.01.16			
		C-5	7 F				
6	Tritium 1.5	HTO-C	F	—	1	27.5	
					2 — Torn.	22.3	
					3	25.4	✓
					4	23.5	
					5	23.4	
					6	27.3	
					7	24.5	
		HTO-D	F	—	1	26.6	
					2 — Torn.	22.8	
					3	22.8	
					4	24.6	✓
					5	26.4	
					6	27.8	
					7	23.0	
		HTO-E	F	—	1	23.2	
					2	25.7	
					3 — Torn.	22.3	
					4	Dead	✓
					5	23.4	
					6	17.9	
					7	23.7	
		HTO-F	F	—	1	24.9	
					2	26.9	
					3 — Torn.	25.9	
					4	22.7	✓
					5	24.0	
					6	23.4	
					7	25.9	

..../3

CRATE	GROUP	CAGE	SEX	DOB	ANIMAL	WEIGHT (gr) AT TREATMENT	
7	Tritium 3.0	HTO-G	M	87.01.15	1	22.8	
					2	20.4	
					3	20.8	✓
					4	22.9	
					5	23.9	
					6	22.3	
					7	21.0	
		HTO-H	M	87.01.16	1	21.6	
					2	18.1	
					3	20.9	
					4	22.1	✓
					5	22.5	
					6	22.9	
					7	24.8	
		HTO-I	F	--	1	21.7	
					2	22.5	
					3	21.5	✓
					4	23.1	
					5	21.2	
					6	22.7	
					7	18.8	
		HTO-J	F	--	1	22.8	
					2 = TORN	22.7	
					3 = TORN	23.1	✓
					4	23.0	
					5	22.3	
					6	22.4	
					7	19.2	

..../4

CRATE	GROUP	CAGE	SEX	DOB	ANIMAL	WEIGHT (gr) AT TREATMENT	
8	Tritium 4.5	HTO-K	M	87.01.16-18			
					1	20.7	
					2 — TORN	21.4	
					3	22.5	
					4	20.9	✓
					5	20.2	
					6	21.8	
					7	23.1	
		HTO-L	M	87.01.19-20			
					1	23.0	
					2	19.5	
					3	23.4	
					4	20.0	✓
					5	20.0	
					6	22.0	
					7	20.2	
		HTO-M	F	—	1	25.6	
					2 — Torn.	26.4	
					3	27.6	✓
					4	21.7	
					5	24.8	
					6	26.0	
					7	25.9	
		HTO-N	F	—	1	21.5	
					2	21.3	
					3	27.3	✓
					4	22.8	
					5	26.9	
					6	25.6	
					7	25.1	

NJG:lm

N.J. Gragtmans

Atomic Energy of Canada Limited
Research Company
Chalk River Nuclear Laboratories

L'Énergie Atomique du Canada, Limitée
Société de Recherche
Laboratoires Nucléaires de Chalk River

Chalk River, Ontario
Canada K0J 1J0
Telephone: 613-584-3311
Telex: 053-34555
Cable Address: "MOTA"
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Physics & Health Sciences

1987 November 30

J. Heddle
Bio-Mutatech Incorporated
4700 Keele Street
Farquharson Building, Room 111
Toronto, Ontario
M3J 1P3

Dear John:

Attached are the individual tritium doses (thanks to the wonders of LOTUS 1-2-3) for the 97 mice we sent you.

The doses were calculated from the injected tritium and mass of each mouse to get the body water concentration on Day 0, and tritium concentration in urine for subsequent days for the next 7 days. These data were then best fitted to a single exponential of the form:

$$C(t) = A\, e^{-\lambda t}$$

The total dose to body water is then given by:

$$D_{BW} = 51.2 \times 0.0057 \times A/\lambda \quad \text{rad}$$

and the average dose to cells was assumed to be 0.733 of this quantity (see attached reference).

With reference to the attached table of doses:

Column 1 — Mouse identification.

Column 2 — Mouse wt at time of injection.

Column 3 — Injected amount.

Column 4 — Initial dose rate. This is not a value used in subsequent calculation. What is used is the concentrations in body water which is calculated by assuming that 60% of the mouse is water.

Column 5 to 8 — Linear regression results.

Column 9 Total dose to body water.

Column 10 Total tissue dose. This is the dose that should be used in the RBE calculations.

Yours sincerely,

JRJ:cy

J.R. Johnson, Manager
Dosimetric Research Branch

Mouse No	Wt (g)	injected (uCi)	Dose rad/d	Linear Regression Data ln(A)	lambda $(Day)^{-1}$	A (uCi) mL^{-1}	Water Dose (rad)	Tissue Dose (rad)	Secondary Slide Code
A1	20.4	4077.0	58.1	5.60	0.38	270	207	152	167
A2	23.7	4077.0	50.0	5.47	0.34	237	203	149	
A3	22.1	4077.0	53.6	5.70	0.36	299	241	177	129
A4	21.3	4077.0	55.7	5.48	0.40	240	174	128	117
A5	24.0	4077.0	49.4	5.37	0.38	215	164	121	154
A6	24.3	4077.0	48.8	5.35	0.38	211	163	119	126
A7	21.3	4077.0	55.7	5.54	0.36	255	206	151	124
B1	20.8	4077.0	57.0	5.47	0.39	237	179	131	160
B2	22.4	4077.0	52.9	5.45	0.36	233	188	138	143
B3	22.2	4077.0	53.4	5.45	0.35	233	196	143	105
B4	20.6	4077.0	57.6	5.44	0.36	230	188	138	
B5	20.9	4077.0	56.7	5.80	0.41	330	234	171	116
B6	18.4	4077.0	64.4	5.53	0.41	252	179	131	
B7	19.6	4077.0	60.5	5.49	0.37	242	193	141	
C1	27.5	4077.0	43.1	5.26	0.30	192	187	137	
C2	22.3	4077.0	53.2	5.38	0.34	217	186	136	59
C3	25.4	4077.0	46.7	5.31	0.33	202	178	131	
C4	23.5	4077.0	50.5	5.29	0.31	198	186	136	
C5	23.4	4077.0	50.7	5.37	0.35	215	179	131	91
C6	27.3	4077.0	43.4	5.30	0.34	200	171	126	
C7	24.5	4077.0	48.4	5.31	0.35	202	168	123	
D1	26.6	4077.0	44.6	5.27	0.23	194	246	180	20
D2	22.8	4077.0	52.0	5.35	0.33	211	186	136	30
D3	22.8	4077.0	52.0	5.40	0.32	221	201	147	47
D4	24.6	4077.0	48.2	5.36	0.30	213	209	153	2
D5	26.4	4077.0	44.9	5.26	0.29	192	193	141	
D6	27.8	4077.0	42.6	5.22	0.30	185	179	131	48
D7	23.0	4077.0	51.6	5.33	0.24	206	250	183	10
E1	23.2	4077.0	51.1	5.52	0.30	250	242	178	5
E2	25.7	4077.0	46.1	5.39	0.30	219	215	158	64
E3	22.3	4077.0	53.2	5.52	0.30	250	242	177	81
E4	found	dead	on	Day 1					
E5	23.4	4077.0	50.7	5.40	0.28	221	231	169	55
E6	17.9	4077.0	66.2	5.51	0.30	247	244	179	72
E7	23.7	4077.0	50.0	5.38	0.29	217	220	161	39

Mouse No	Wt (g)	injected (uCi)	Dose rad/d	Linear Regression Data ln(A)	lambda $(Day)^{-1}$	A (uCi) mL^{-1}	Water Dose (rad)	Tissue Dose (rad)	Secondary Slide Code
F1	24.9	4077.0	47.6	5.23	0.30	187	181	133	66
F2	26.9	4077.0	44.1	5.28	0.31	196	184	135	40
F3	25.9	4077.0	45.8	5.28	0.32	196	180	132	85
F4	22.7	4077.0	52.2	5.35	0.36	211	168	123	1
F5	24.0	4077.0	49.4	5.35	0.29	211	211	155	77
F6	23.4	4077.0	50.7	5.33	0.31	206	194	142	79
F7	25.9	4077.0	45.8	5.36	0.29	213	216	159	74
G1	22.8	8153.0	104.0	6.11	0.38	450	347	255	170
G2	20.4	8153.0	116.2	6.12	0.36	455	367	269	161
G3	20.8	8153.0	114.0	6.12	0.36	455	373	273	104
G4	22.9	8153.0	103.5	6.07	0.35	433	355	260	149
G5	23.9	8153.0	99.2	6.03	0.34	416	358	262	
G6	22.3	8153.0	106.3	6.08	0.37	437	345	253	130
G7	21.0	8153.0	112.9	6.13	0.35	459	380	278	125
H1	21.6	8153.0	109.8	6.13	0.36	459	375	275	110
H2	18.1	8153.0	131.0	6.27	0.36	528	431	316	141
H3	20.9	8153.0	113.4	6.19	0.34	488	419	307	
H4	22.1	8153.0	107.3	6.15	0.35	469	395	290	173
H5	22.5	8153.0	105.4	6.08	0.32	435	390	286	
H6	22.9	8153.0	103.5	6.00	0.36	403	328	240	136
H7	24.8	8153.0	95.6	6.02	0.34	412	349	256	
I1	21.7	8153.0	109.3	6.16	0.27	473	520	381	94
I2	22.5	8153.0	105.4	6.15	0.29	469	472	346	32
I3	21.5	8153.0	110.3	6.27	0.31	528	499	366	52
I4	23.1	8153.0	102.6	6.16	0.27	473	512	375	17
I5	21.2	8153.0	111.8	6.13	0.24	459	557	408	95
I6	22.7	8153.0	104.5	6.17	0.27	478	523	384	92
I7	18.8	8153.0	126.1	6.32	0.25	556	647	474	69
J1	22.8	8153.0	104.0	6.15	0.23	469	603	442	50
J2	22.7	8153.0	104.5	6.15	0.29	469	475	348	65
J3	23.1	8153.0	102.6	6.13	0.27	459	495	363	22
J4	23.0	8153.0	103.1	6.17	0.28	478	497	364	90
J5	22.3	8153.0	106.3	6.18	0.27	483	516	379	45
J6	22.4	8153.0	105.8	6.17	0.27	478	515	378	93
J7	19.2	8153.0	123.5	6.23	0.27	510	552	405	62
K1	20.7	12230.0	171.8	6.57	0.31	711	671	492	150
K2	21.4	12230.0	166.2	6.61	0.34	739	623	457	119
K3	22.5	12230.0	158.1	6.50	0.37	664	529	388	101
K4	20.9	12230.0	170.2	6.52	0.34	679	589	432	145
K5	20.2	12230.0	176.1	6.64	0.32	768	707	518	179
K6	21.8	12230.0	163.2	6.55	0.34	699	600	440	176
K7	23.1	12230.0	154.0	6.46	0.32	637	575	422	177

Mouse No	Wt (g)	injected (uCi)	Dose rad/d	Linear Regression Data ln(A)	lambda $(Day)^{-1}$	A (uCi) mL^{-1}	Water Dose (rad)	Tissue Dose (rad)	Secondary Slide Code
L1	23.0	12230.0	154.6	6.49	0.36	659	532	390	164
L2	19.5	12230.0	182.4	6.60	0.34	736	630	462	178
L3	23.4	12230.0	152.0	6.43	0.34	620	532	390	156
L4	20.0	12230.0	177.8	6.63	0.37	757	588	431	171
L5	20.0	12230.0	177.8	6.55	0.35	699	578	424	138
L6	22.0	12230.0	161.7	6.49	0.37	656	515	378	108
L7	20.2	12230.0	176.1	6.61	0.36	742	607	445	139
M1	25.6	12230.0	138.9	6.43	0.31	620	582	426	44
M2	26.4	12230.0	134.7	6.45	0.31	633	598	438	
M3	27.6	12230.0	128.9	6.36	0.31	577	541	396	
M4	21.7	12230.0	163.9	6.64	0.27	765	824	604	
M5	24.8	12230.0	143.4	6.47	0.35	645	533	391	
M6	26.0	12230.0	136.8	6.46	0.27	639	701	514	68
M7	25.9	12230.0	137.3	6.22	0.31	503	476	349	71
N1	21.5	12230.0	165.4	6.64	0.29	765	771	565	27
N2	21.3	12230.0	167.0	6.51	0.23	672	839	615	80
N3	27.3	12230.0	130.3	6.31	0.20	550	804	589	18
N4	22.8	12230.0	156.0	6.42	0.27	614	661	485	31
N5	26.9	12230.0	132.2	6.46	0.25	637	753	552	76
N6	25.6	12230.0	138.9	6.34	0.18	568	919	674	3
N7	25.1	12230.0	141.7	6.53	0.25	685	807	592	75

Atomic Energy of Canada Limited
Research Company
Chalk River
Nuclear Laboratories

L'Énergie Atomique du Canada, Limitée
Société de Recherche
Laboratoires Nucléaires de Chalk River

Chalk River, Ontario
Canada K0J 1J0
Telephone: 613-584-3311
Telex: 053-34555
Cable Address: "MOTA"
Telecopier: 613-589-2039
Word Processor Data Link: 613-589-2218

Physics & Health Sciences

1988 February 8

E. Rabin
Atomic Energy Control Board
270 Albert Street
P.O. Box 1046
Ottawa, Ontario
K1P 5S9

Dear Eric:

Below are given the X-ray doses delivered in the cytogenetic experiment.

Rack	Nominal Dose (Gy)	Actual Dose (Gy)
A	1.5	1.33 ± 0.01
B	3.0	2.82 ± 0.03
C	4.5	3.36 ± 0.18

Yours sincerely,

Christine Ipaktchi

JRJ:cy

for J.R. Johnson, Manager
Dosimetric Research Branch

APPENDIX XII

CYTOGENETIC MEASUREMENTS OF THE RELATIVE BIOLOGICAL EFFECTIVENESS OF TRITIUM: A STATISTICAL REANALYSIS

September 1, 1988

Barry H. Margolin, Ph.D.
Susan A. Murphy, M.S.

INTRODUCTION

This report presents the results of an extensive statistical reanalysis of the data from Phase II of the study described in "Cytogenetic Measurements of the Relative Biological Effectiveness of Tritium" by Bio-Mutatech. The primary objective of this reanalysis is the development of both the best estimates of the relative biological effectiveness of tritium compared to X-rays and the statistical uncertainty associated with these estimates. The report has five components:

(a) Quality control checking of the primary database needed for the reanalysis and a review of the initial analysis ("the preliminary analysis");

(b) Exploration of the appropriate weights to be employed in the reanalysis for any endpoint for each individual animal;

(c) Estimation of the relative biological effectiveness of tritium to X-rays based on a weighted regression analysis of a linear dose response model for each endpoint;

(d) Construction of interval estimates for the relative biological effectiveness of tritium compared to X-rays; and

(e) Discussion.

QUALITY CONTROL CHECKING OF DATA AND REVIEW OF PRELIMINARY ANALYSIS

The translocation and chromosome aberration data analyzed in this report are found in various tables in the Bio-Mutatech report cited above. These data were only available in a typed format, so the initial step of the reanalysis was creation of a computerized database. Data and treatment doses were keyed into computer memory and listings from this database were hand-checked against the primary tabular data presented in the Bio-Mutatech report. The keyed data were also compared to the listings of data that constituted the input to the

linear regression computations in the preliminary analysis. In this process, three minor inconsistencies were discovered between the Bio-Mutatech tabular presentations and the regression inputs for translocations:

Table No.	Animal Code	Slide	Dose	Tabular Data	Regression Data
25	256	131	1.5 Gy X-ray	5	3
26	272	155	3.0 Gy X-ray	3	4
27	275	152	4.5 Gy X-ray	2	3

The assumption was made that the tabular data presentations in the Bio-Mutatech report were correct. The perturbations introduced into the regression analyses by the errant data were judged to be of little significance.

Of slightly more importance was the fact that the proposed formula differed from the one actually used in the preliminary analysis. The computations in the preliminary report omitted the subtraction of overall means from both independent or dose (X) and dependent or response (Y) variables, which resulted in regression estimates appropriate to the linear model

$$Y = aX,$$

i.e., a linear dose response with a postulated zero intercept. The model proposed in the preliminary analysis and the one adopted here is

$$Y = aX + b, \qquad (1)$$

which allows for a positive spontaneous rate for each of the endpoints considered.

EXPLORATION OF THE APPROPRIATE REGRESSION WEIGHTS

The preliminary analysis contained a weighted regression analysis of the individual animal means, with weights equal to the number of cells scored per

animal; this gives appropriately greater weight to animals for whom more cells were scored. This weighting scheme, however, neglects other considerations that need to be taken into account in the weighted regression.

A weighted regression analysis is appropriate because the individual animal means have different variances. The weights that constitute the least squares (or maximum likelihood if normality is assumed) regression estimates are inversely proportional to the corresponding variances. The key, then, to the weights is an accurate assessment of the variances of individual animal means (Y_i). If n_i cells were scored for the i^{th} animal, then the variance of the mean Y_i is composed of an inter-animal component (σ^2_{animal}) and an intra-animal inter-cellular component (σ^2_{cell}), i.e.,

$$var(Y_i) = \sigma^2_{animal} + \frac{1}{n_i}\sigma^2_{cell} \quad . \qquad (2)$$

Furthermore, both components of variability can change with dose. The preliminary analysis simply assumed that

$$var(Y_i) = \frac{1}{n_i}\sigma^2_{cell}$$

and that the cellular component of variability σ^2_{cell} was essentially constant across doses. Because the endpoints being scored are rare events, the cellular component of variability will increase with the mean, so that in the present context observations at higher doses are more variable than observations at lower doses for equal numbers of cells scored.

As a preliminary step in the reanalysis, an assessment was made of the applicability of a common Poisson model for animals exposed to a common dose of X-rays. In this context, the binomial model is essentially equivalent to the Poisson. The tritium-treated animals present an additional complexity — animals within a common treatment group received differing doses, so that an

assessment of Poisson sampling is intertwined with the shape of the dose response over the relevant range of doses within a treatment group. Table 1 contains the test of the applicability of the Poisson/binomial sampling model to the X-ray data. For both terminal deletions and translocations, there is evidence of inflated variability at the highest two doses, with the estimated inter-animal variability being approximately twice that suggested by the Poisson/binomial model. For the different rings and interstitial deletions, the Poisson/binomial model offers acceptable descriptions of the sampling variability for the number of cells scored.

TABLE 1. APPLICABILITY OF A POISSON/BINOMIAL MODEL FOR X-RAY DATA

Endpoint	Dose	Mean	Dispersion[1] Test	P-Value[2]	Variance Inflation[3] Factor
Dicentric & Centric Rings	0	0	NM[4]	NM	NM
	1.33	0.024	21.16 (19)[5]	0.33	1.11
	2.82	0.043	14.61 (9)	0.10	1.61
	3.36	0.052	11.52 (9)	0.24	1.28
Terminal Deletions	0	0.001	9.01 (9)	0.43	1.00
	1.33	0.054	30.52 (19)	0.05	1.61
	2.82	0.079	17.99 (9)	0.04	2.00
	3.36	0.102	17.23 (9)	0.05	1.91
Interstitial Deletions & Acentric Rings	0	0.002	8.02 (9)	0.53	0.89
	1.33	0.010	15.89 (19)	0.67	0.84
	2.82	0.013	9.43 (9)	0.40	1.05
	3.36	0.014	8.84 (9)	0.45	0.98
Translocations	0	0.001	11.02 (14)	0.69	0.79
	1.33	0.013	11.54 (9)	0.24	1.28
	2.82	0.015	17.71 (9)	0.04	1.97
	3.36	0.023	17.85 (9)	0.04	1.98

[1]The common test for Poisson or binomial sampling when events are rare is given by a disperson test (see Margolin et al, 1986).

[2]P-value associated with dispersion test.

[3]Ratio of estimated variance among animals to that postulated by Poisson/binomial model.

[4]Not meaningful.

[5]Degrees of freedom.

In light of the sizeable number of animals per dose group ($\geq$ 10), the decision was made to use empirical estimates of the inter-animal and intra-animal inter-cellular components of variability for each dose group. This is most readily accomplished by performing separate one-way analyses of variance for each endpoint and dose group, with the individual cell being the unit of measurement. Between and Within Animal mean squares can be set equal to their respective expected values and the resulting two equations solved for least squares estimates of σ^2_{animal} and σ^2_{cell} (see Graybill, 1961, p. 351). This computation is performed separately for each treatment, endpoint and dose group, a total of 28 analyses of variance in all.

ESTIMATION OF THE RELATIVE BIOLOGICAL EFFECTIVENESS OF TRITIUM TO X-RAYS

The estimates of σ^2_{cell} and σ^2_{animal} obtained from the 28 analyses of variance, when substituted into Eq. 2, yield treatment-endpoint-dose-specific estimates of the variance of the corresponding means Y_i based on n_i cells scored. The appropriate least squares regression weights are then the reciprocals of these estimated variances. The general result is that lower dose data are given proportionately greater weight than they would in an unweighted regression, reflecting their comparatively lower inter-animal and intra-animal inter-cell variability. Regression analyses initially were computed separately for each endpoint and each treatment. This allowed a check on whether the regression error mean squares ("the error") for tritium and X-rays were approximately equal for each endpoint. The evidence in Table 2 strongly supports this equality (after adjustment for possible second-order curvature of the dose response).

Based on the near equality of the tritium and X-ray error mean squares, a combined regression analysis for the two treatments was performed for each of

TABLE 2. REGRESSION MEAN SQUARE ERRORS

Endpoint	Tritium Mean Square Error	X-Ray Mean Square Error
Dicentric & Centric Rings	79.0 (51)[a]	80.4 (46)
Terminal Deletions	106.1 (51)	100.0 (46)
Interstitial Deletions & Acentric Rings	94.4 (51)	90.6 (46)
Translocations	92.8 (46)	92.7 (41)

[a]The number in parentheses represents the degrees of freedom associated with the appropriate regression mean square error.

the four endpoints. The model for each treatment was that in Eq. 1. For each endpoint, a common intercept was assumed for the tritium and X-ray linear dose responses, since this reflects the single common spontaneous rate. The weighted regressions yield estimates of the slopes of the dose response for tritium and X-ray, which are presented in Table 3. This table also contains estimates of the relative biological effectiveness (RBE) of tritium to X-rays.

TABLE 3. WEIGHTED REGRESSION SLOPE ESTIMATES AND RELATIVE BIOLOGICAL EFFECTIVENESS (RBE) OF TRITIUM TO X-RAYS

Endpoint	Tritium Regression Slope	X-Ray Regression Slope	RBE	95% Confidence Interval for RBE[a]
Dicentric & Centric Rings	1.82 (0.20)[b]	1.60 (0.18)	1.14	(0.85, 1.53)
Terminal Deletions	3.02 (0.23)	3.20 (0.29)	0.94	(0.75, 1.20)
Interstitial Deletions & Acentric Rings	0.61 (0.12)	0.41 (0.10)	1.49	(0.85, 2.92)
Translocations	0.76 (0.12)	0.63 (0.10)	1.21	(0.77, 1.94)

[a]Obtained from Fieller's Theorem (see Finney, 1978, p. 80).

[b]Estimated standard error of corresponding slope estimate.

Fitting a model that relaxed the assumption of a common intercept altered no RBE value by more than 0.02, an insignificant change given the uncertainty attached to each estimated RBE.

CONSTRUCTION OF INTERVAL ESTIMATES OF THE RBE FOR TRITIUM VERSUS X-RAY

The point estimates of the RBE for tritium compared to X-rays in Table 3 can only be adequately interpreted in light of their corresponding statistical uncertainties. A standard method used to obtain confidence limits for an RBE is to apply Fieller's Theorem (Finney, 1978, p. 80). The limits obtained from this theorem are included in Table 3. Interestingly, one cannot exclude an RBE of unity as a plausible value for any of the four endpoints. At first glance, the confidence limits may strike one as unduly wide, e.g., that for interstitial deletions and acentric rings stretches from 0.85 to 2.92, a range of more than two units. A better appreciation of this width can be derived from considering the slope estimates that constitute the RBE and their individual uncertainties. For example, the tritium regression slope of 0.61 is associated with an 0.12 standard error, while the X-ray slope is 0.41 with an 0.10 standard error. It then follows that an X-ray slope could easily be as low as 0.25. Such a value, when linked to the observed tritium slope estimate of 0.61, yields 2.4 as a plausible value for the true RBE. This phenomenon of broad confidence limits for ratios is well known to occur when the denominator is small relative to its standard error, as is the case with interstitial deletions and acentric rings and translocations. The confidence limits are much tighter for dicentric and centric rings and terminal deletions, which have smaller coefficients of variation, i.e., ratios of the standard error to the regression slope.

DISCUSSION

In general, the assessment of an RBE rests heavily on an assumption of linearity for the two dose responses being compared. If linearity of response within the range of experimentation holds for both X-rays and tritium, then the RBE values and their confidence limits in Table 3 are valid as presented. Extrapolation of an RBE to doses beyond the common range of experimentation, however, would have to be based on biological justification. On the other hand, non-linearity, if present in one or both dose responses being compared, would not entirely invalidate the associated RBE estimate; this would depend on the degree of non-linearity present in the relevant portion of the dose range.

For the present data, there is a suggestion of dose response curvature for terminal deletions at the highest dose of tritium, although there is not sufficient precision in the data to permit the drawing of a firm conclusion on this point. A similar caution applies to translocations. Depending upon the intended use of the estimated RBE, further experimental work may be needed to refine this estimate. Alternatively, biological theory may be able to resolve the issue of possible curvature directly.

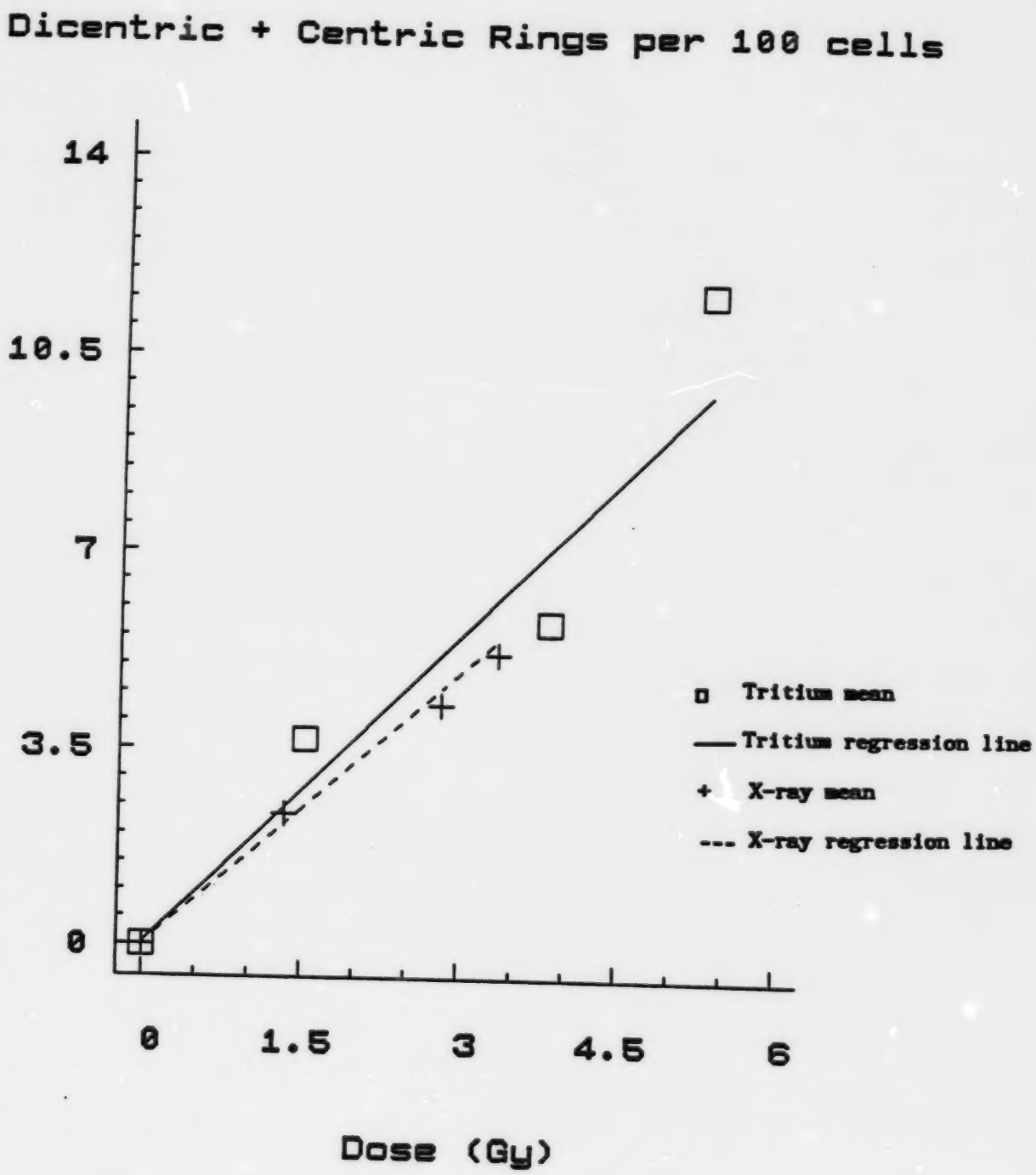
Finally, one additional general comment about possible further experimentation is in order. The design of any subsequent experiment with the current four endpoints or other related experiments should build upon the results of the current study. Based on the estimates of inter-animal and intra-animal components of variability presented here, one should be able to design experiments in which the number of doses, the number of animals per dose and the number of cells per animal are determined to enable efficient estimation of the biological parameters of interest.

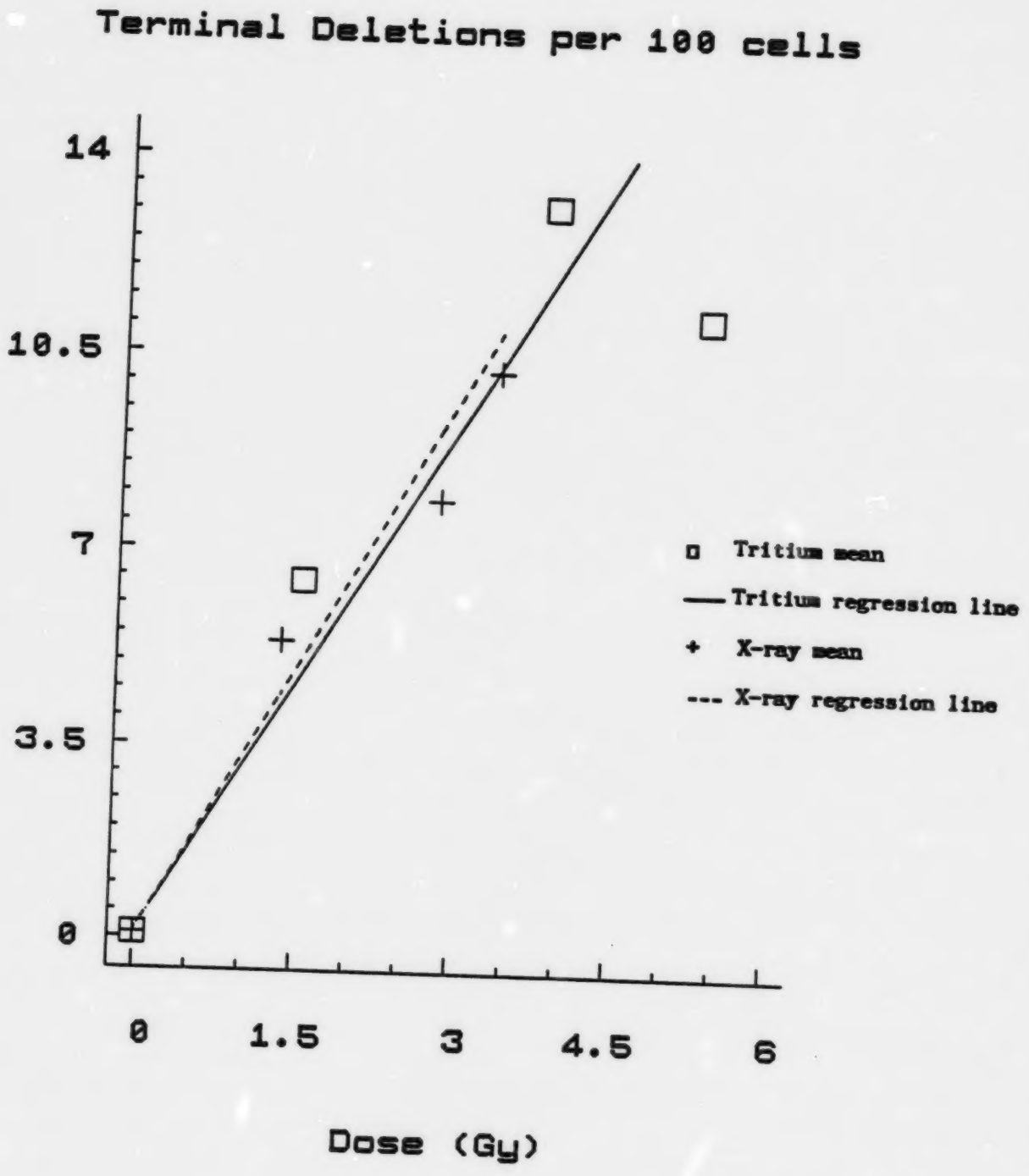
REFERENCES

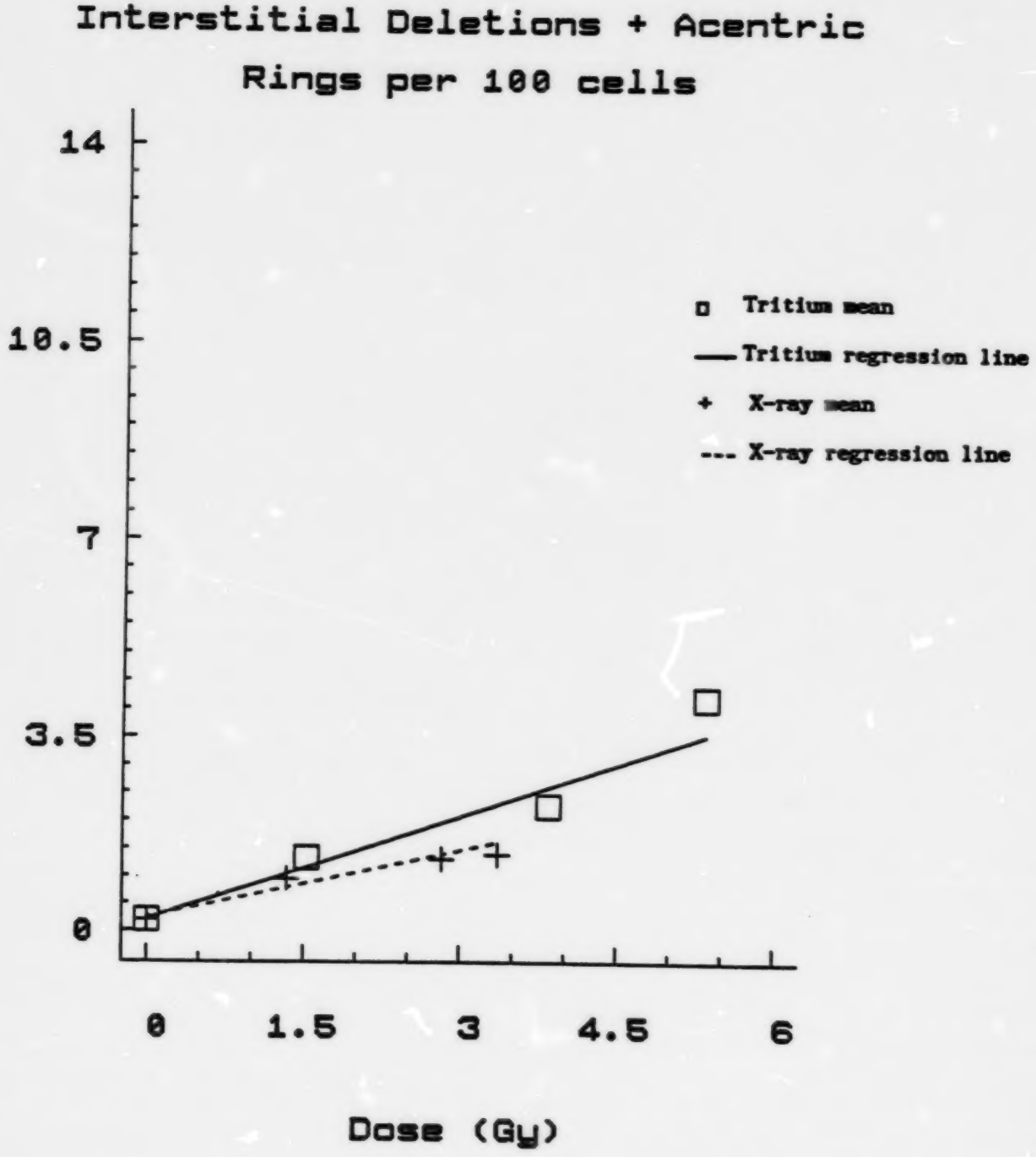
Finney, D.J. (1978). *Statistical Method in Biological Assay*. London: Charles Griffin & Co.

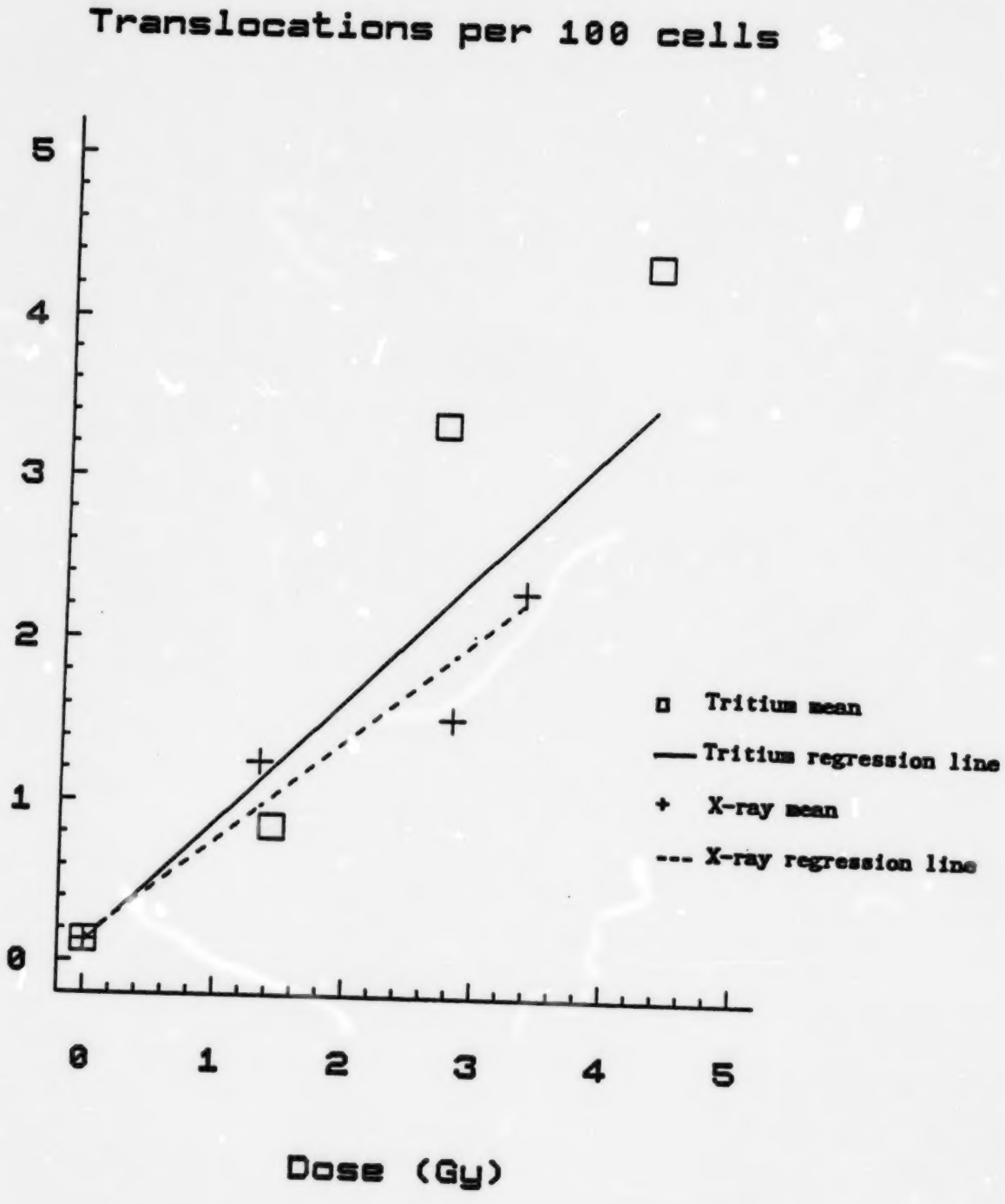
Graybill, F.A. (1961). *An Introduction to Linear Statistical Models*. New York: McGraw-Hill Co.

Margolin, B.H., et al. (1986). Statistical analyses for in vitro cytogenetic assays using Chinese hamster ovary cells. *Environmental Mutagenesis* 8: 183-204.


Dicentric + Centric Rings per 100 cells
14
10.5
7
3.5
0
0
1.5
3
4.5
6
Dose (Gy)
Tritium mean
Tritium regression line
X-ray mean
X-ray regression line


Terminal Deletions per 100 cells
14
10.5
7
3.5
0
0
1.5
3
4.5
6
Dose (Gy)
Tritium mean
Tritium regression line
X-ray mean
X-ray regression line


Interstitial Deletions + Acentric
Rings per 100 cells
14
10.5
7
3.5
0
0
1.5
3
4.5
6
Dose (Gy)
Tritium mean
Tritium regression line
X-ray mean
X-ray regression line


Translocations per 100 cells
5
4
3
2
1
0
0
1
2
3
4
5
Dose (Gy)
Tritium mean
Tritium regression line
X-ray mean
X-ray regression line

APPENDIX XIII

DOSIMETRY FOR THE MYELOID LEUKEMIA TRITIUM RBE STUDY

by

J.R. Johnson

and

A.R. Jones

Dosimetric Research Branch
Chalk River Nuclear Laboratories
Chalk River, Ontario K0J 1J0

Prepared for the Atomic Energy Control Board Panel

1988 February

Introduction

The experimental protocol for this study has been discussed in detail elsewhere (INFO-0175, Rad. Prot. Dosim. 16 161). As stated in these reports, 5000 young male mice were required to carry out the study. The mice for the study were bred at CRNL from stock obtained from Harwell. Their genetic profile has been certified to be consistent with the CBA/H strain by Charles River. This report briefly describes the irradiations and dosimetry used in this study.

The animal facilities at CRNL are not large enough to maintain a mouse colony large enough to produce 5000 male mice all at the same age; nor are the irradiation facilities large enough to irradiate all of the animals at one time. The colony was therefore bred up to a size thought to be capable of producing a set of about 850 male mice every two weeks. This would allow us to do the irradiations in 6 sets, one every two weeks. However, the mice did not breed as fast as anticipated and, in addition, problems with the X-ray set also caused delays, and these combined problems resulted in there being 9 sets of mice irradiated, some by HTO only, some by X-rays only, and some by both.

As mentioned above, the X-ray set failed part way through the irradiation. This necessitated a change from a 300 kVcp set (run at 200 kVcp) to a 150 kVcp set. This change in voltage will result in a change in the photon energy spectrum, which has the potential to change the average LET of the X-rays used in the irradiations. Absorbers were used in order to keep the average LET the same for the two X-ray sets, as explained later.

Tritium Dosimetry

The tritium (as HTO) used in the experiment was obtained from Amersham and calibrated as described in Attachment 1. The working standard mentioned in Attachment 1 is the CRNL standardized HTO that is traceable to an NBS standard. Mice were injected with 0.2 mL, 0.4 mL and 0.6 mL of the HTO in order to deliver a dose of approximately 1, 2 and 3 Gy respectively. Mice were then maintained in cages in a vented rack (Fig. 1) and urine was collected from mice in selected cages (see Table 1) for about 2 weeks post injection. These urine samples were analyzed for tritium concentration by first diluting the samples and then liquid scintillation counting. Standard methods of quench corrections, calibrations, etc. were used. (It should be noted that the method used at CRNL for tritium in urine analyses is cross-calibrated with other Canadian laboratories as part of the inter-comparison program run by Health & Welfare Canada).

The measured tritium concentrations were entered into a computer (LOTUS 1-2-3) as shown in Attachment 2. These data were then fitted to a single exponential of the form:

$$C(t) = A\, e^{-\lambda t}$$

The total dose to body water is then given by:

$$D_{BW} = 51.2 \times 0.0057 \times A/\lambda \text{ rad}$$

and the average dose to cells was assumed to be 0.733 of this quantity (see Rad. Prot. Dosim. 2 245 (1982)). This factor of 0.733 is based on data reviewed in NCRP Report No. 63. Resulting doses are given in Table 2.

X-Ray Dosimetry

Cages containing the mice were positioned in three racks as shown schematically in Fig. 2. Initial calibrations were done by inserting TLD's subcutaneously in mice in the cages, and relating these doses to the doses measured by TLD's positioned on the outside of cages as shown in Fig. 2. (This had to be repeated for the 150 kVcp set.) TLD's at these positions during the actual irradiations were used to assign average doses to sets of mice that are given in Table 3.

The doses delivered to the animals were reduced by approximately one half every two days for a period of 10 days. These reductions were achieved by using an ion chamber which displayed a count rate and accumulated counts. This ion chamber had previously been calibrated against the TLD results on the cages.

Typical results for the control ion chamber are shown below.

Two-day Period	Count Rate (/s)	Accumulated Counts
First	547	9.44E7
Second	300	5.17E7
Third	164	2.83E7
Fourth	91	1.56E7
Fifth	50	8.77E6

Because the HV X-ray set, which had previously been operated at 200 kV had failed, it was necessary to use a 150 kV machine (also operated at constant potential). To increase the average energy of the spectrum an ISO filter designed to produce a more uniformly monoenergetic spectrum was used. This filter will produce an X-ray spectrum with an average energy of 104 keV, which is approximately equal to the average energy of the spectrum from the 200 kVcp set (see Fig. 3). These spectra will produce primarily electrons in tissue that have essentially equal average energies, and hence the average LET will be essentially the same for the two X-ray potentials used in the study.

The statement that the average energy of the primary electrons produced in tissue from the two different X-ray sets are similar, and that these produce in LET that essentially the same is supported by the work of Todo et al. (Health Physics 43 845-852 (1982)). That report gives the energy spectra

of the primary electrons produced by monoenergetic photons at 10 keV intervals from 10 to 100 keV photon energies, and 20 keV intervals from 100 to 200 keV photon energies. A copy of this data is given below.

Initial electron-energy distribution in water (first-collision spectrum). Number of electrons/cm^{-1} · keV per photon/cm^{2}

Electron Energy Interval (keV)	Photon Energy (keV)															
	10	20	30	40	50	60	70	80	90	100	120	140	160	180	200	220
0-5	201	108	64.2	47.1	31.7	24.7	20.6	17.6	15.2	13.6	11.3	9.58	8.44	7.63	7.03	6.61
5-10	108	8	0	3.79	13.8	12.2	8.60	6.97	6.13	5.22	4.27	3.37	2.89	2.78	1.88	1.47
10-15		0	0	0	0	5.46	11.6	6.73	5.08	4.48	3.53	2.84	2.44	2.08	1.75	1.51
15-20		70.2	0	0	0	0	0.202	6.02	5.05	4.11	3.08	2.58	2.13	1.78	1.57	1.37
20-25			0	0	0	0	0	0	5.91	5.16	2.90	2.34	1.97	1.72	1.42	1.28
25-30			21.8	0	0	0	0	0	0	4.91	3.11	2.16	1.79	1.61	1.41	1.20
30-35				0	0	0	0	0	0	0	3.87	2.19	1.67	1.46	1.32	1.17
35-40				8.82	0	0	0	0	0	0	3.52	2.40	1.67	1.36	1.21	1.11
40-45					0	0	0	0	0	0	0	2.97	1.77	1.29	1.13	1.03
45-50					4.17	0	0	0	0	0	0	3.55	1.91	1.32	1.08	1.00
50-55						0	0	0	0	0	0	0	2.29	1.39	1.08	0.935
55-60						2.38	0	0	0	0	0	0	2.79	1.56	1.10	0.913
60-65							0	0	0	0	0	0	1.16	1.00	1.10	0.912
65-70							1.40	0	0	0	0	0	0	2.15	1.27	0.914
70-75								0	0	0	0	0	0	2.28	1.61	0.974
75-80								0.919	0	0	0	0	0	0	1.63	1.04
80-85								0	0	0	0	0	0	0	1.94	1.17
85-90									0.881	0	0	0	0	0	1.27	1.24
90-95	MULTIPLY TABLE VALUES BY 10^{-3}									0	0	0	0	0	0	1.51
95-100										0.341	0	0	0	0	0	1.72
100-110											0	0	0	0	3	0.360
110-210											0.00999	0.00563	0.00253	0	0	0
Average Number of Electrons/cm^{2} per Photon/cm^{2}	1.96	0.891	0.430	0.298	0.248	0.224	0.211	0.200	0.194	0.187	0.175	0.171	0.166	0.158	0.153	0.149

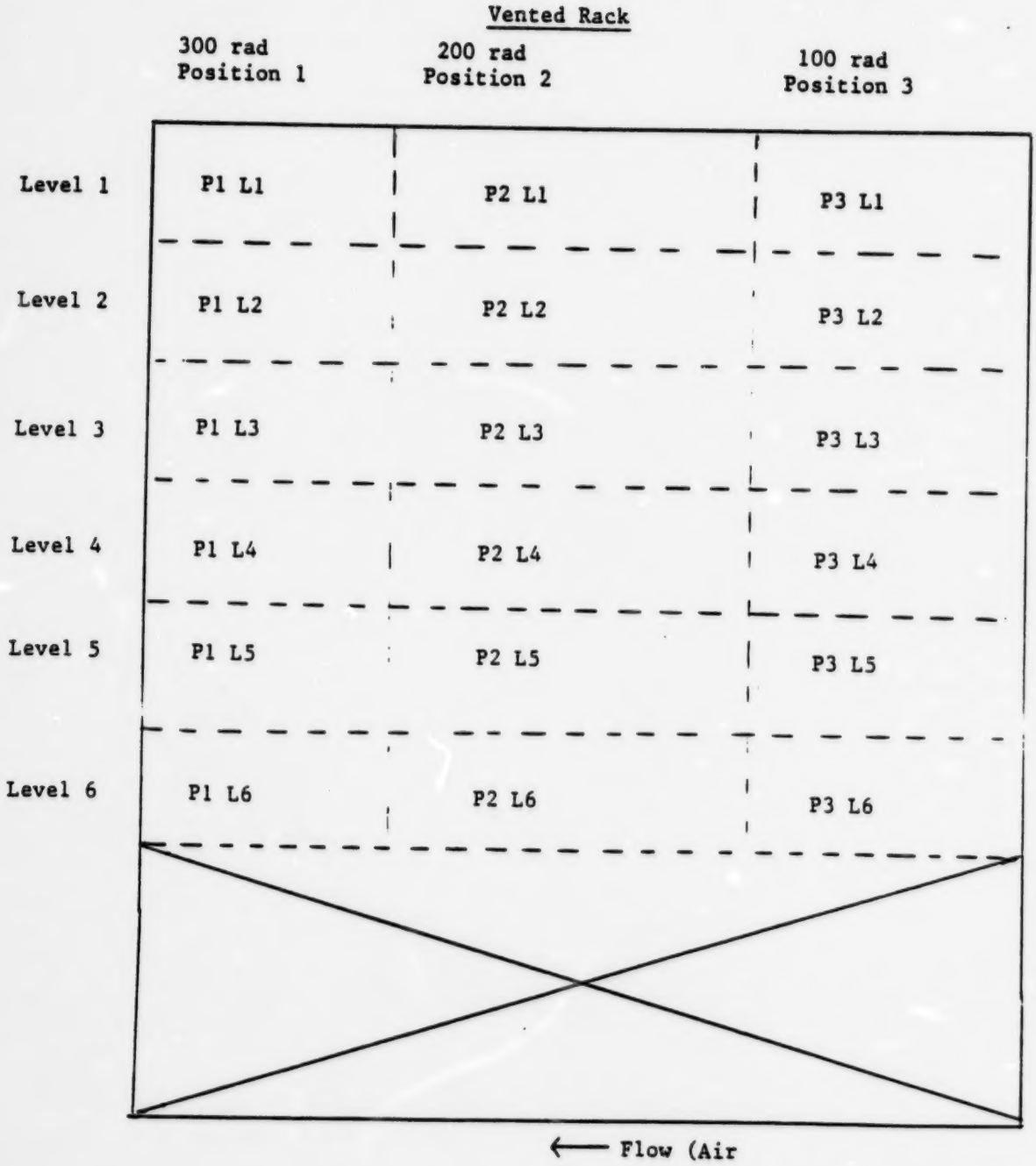
The primary difference in the two X-ray spectra is that the 200 kVp spectra contains photons between 150 and 200 keV that are not in the 150 kVp spectrum, and the intensity of photons between about 40 keV and 60 keV is higher for the 200 kVp spectrum than for the 150 kVp spectrum. In addition the intensity of photons in the 60 to 140 keV range is higher in the 150 kVp spectra than in the 200 kVp spectrum. While a detailed calculation has not been done, it is apparent that the production of primary electrons in energy bands 0 - 5 keV and 5 - 10 keV (which have the most effect on the LET) will be about the same for the two spectra.

To obtain the necessary range of dose rates (11:1) acrylic attenuators of varying thicknesses were needed. Also the arrangement of cages necessitated some difference in the direction of the X-ray beam. Pulse height spectra taken with attenuators varying between 76 and 178 mm and in direction between 0 and 10 degrees did not differ significantly indicating that the average LET would not vary with attenuation or beam direction.

Data Storage

The doses in Tables 2 and 3 are part of the information in the MICE database maintained by the Radiation Biology Branch on an IBM XT. This database also contains the date of birth ($\pm$ 1 day) and date of irradiation of each mouse in the study. The pathology and histology data are being put into the database as it is accumulated.

FIGURE 1



3 cages at each rack position.

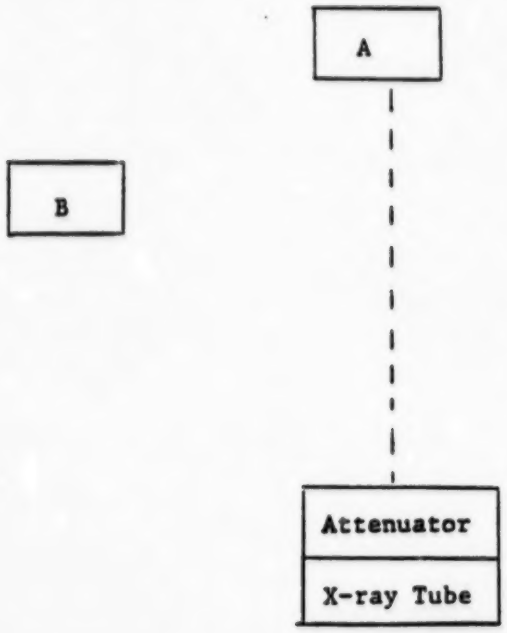
ARRANGEMENT OF CAGES

1*	7
2	8*
3*	9
4*	10*
5*	11
6	12*

*cages with TLDs

Not to scale.

Rack POSITIONS



Not to scale

FIGURE 3: Comparison of the 150 and 200 kVp spectra.

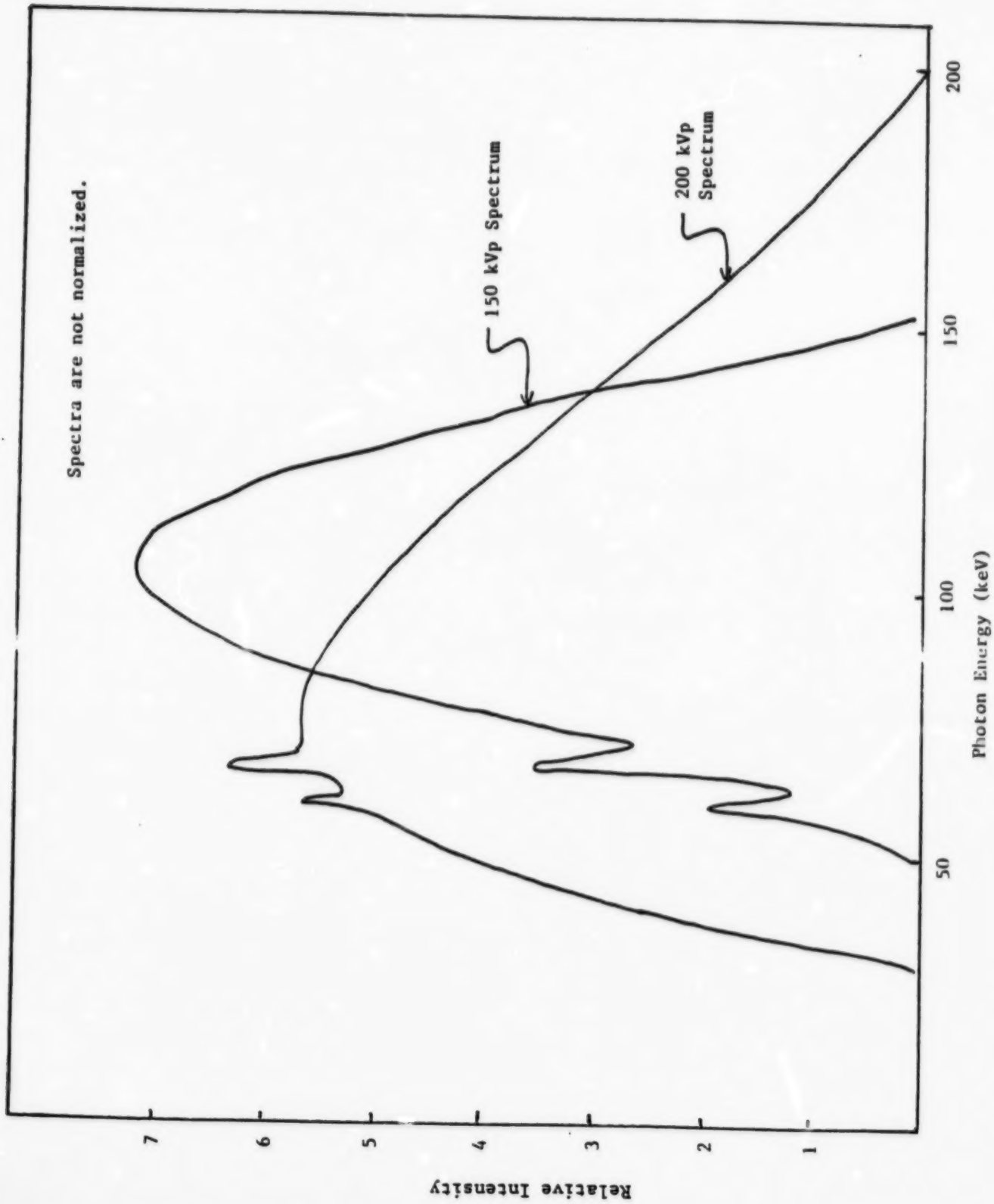


TABLE 1: Typical sampling schedule for tritium in urine.

Day 1	One cage from each of:
	P1L1, P2L1, P3L1, P1L2, P2L2, P3L2
Day 2	P1L3, P1L4, P2L3, P2L4, P3L3, P3L4
Day 3	P1L5, P1L6, P2L5, P2L6, P3L5, P3L6
Day 4	Day 1 schedule
Day 5	Day 2 schedule
Day 8	Day 3 schedule
Day 9	Day 1 schedule
Day 10	Day 2 schedule
Day 11	Day 3 schedule

etc.

TABLE 2: Tritium sets.

Set	Date of Injection	Dose (Gy) HTO1	HTO2	HTO3
1 (1)	86/09/29	0.87	1.99	2.93
2 (2)	86/10/27	0.84	1.81	3.06
3 (3)	86/11/10	0.88	1.70	2.93
3a(4)	86/11/17	0.82	1.64	2.59
6 (5)	86/12/18	0.84	1.91	3.02
8 (6)	87/01/08	0.86	2.11	3.67

TABLE 3: X-ray sets.

Set	Treatment Date (1)	Dose (Gy) XRAY1	XRAY2	XRAY3	Ref. (2)
1	86/09/29	1.04	2.31	3.13	1986/10/15
5	86/11/21	0.92	1.92	2.32	1986/12/17
6	86/12/18	1.15	1.91	2.61	1987/02/23
7	86/12/28	1.05	1.87	2.51	1987/02/23
8	87/01/08	1.02	1.79	2.43	1987/02/23
9	87/01/23	1.20	2.13	2.87	1987/02/23

(1) Date of Start of Treatment
(2) Date of Memorandum from A.R. Jones

ATTACHMENT #1

TRITIUM CALIBRATION 11-9-86

A tritium solution was obtained from Amersham. The nominal amount was 20 Ci in a volume of 4 mL. The solution was standardised in the following way:

0.1365 g diluted to 49.946 g - this is solution A

0.1008 g of A diluted to 49.9086 - this is solution B

0.1013 g of B diluted to 49.6383 - this is solution C

Three aliquots (0.5 mL) of solution C were added to 4 mL of Scintiverse and counted for several cycles.

Three aliquots (0.5 mL) of a working standard (305.01 µCi/L) were added to 4 mL of Scintiverse and counted for several cycles. The quenching in the two sets of samples was found to be the same so that the concentration of C could be obtained directly for the working standard. The data is shown below.

Sample	Average Count Rate	Average	Concentration uCi/L
C1	21198		
C2	21523	21467	50.5
C3	21680		
S1	128847		
S2	129448	129659	305.01
S3	130683		

Therefore:

Solution B = 50.5 ✕ 490.013 = 24745.17 µCi/L
= 9.16 E5 Bq/mL

Solution A = 9.16 E5 ✕ 495.125 = 4.53 E8 Bq/mL

Solution A was made 0.9% NaCl by adding 0.45 g NaCl to the solution.

TRITIUM SOLUTIONS FOR MAIN EXPERIMENT

Three solutions were prepared in a similar manner to that described above. The results are presented below:

Solution A. 0.1629 g of Tritium Soln. diluted to 59.9969 g.
Solution B. 0.1627 g of Tritium Soln. diluted to 59.9964 g.
Solution C. 0.1632 g of Tritium Soln. diluted to 59.9994 g.

The resulting concentrations are, therefore as follows:

Solution A. 4.50 E8 Bq/mL
Solution B. 4.49 E8 Bq/mL
Solution C. 4.51 E8 Bq/mL

ATTACHMENT #2
Sample Data from Set No. 1

Mice set No. 1
Date of injection 86/9/29

Position	Level	Time	Conc.	Ln (conc)	Time	Avg (conc)
p1	11	1	291.00	5.67	1	295.35
p1	12	1	299.70	5.70		
p1	14	2	210.90	5.35	2	213.50
p1	13	2	216.10	5.38		
p1	16	3	149.20	5.01	3	125.35
p1	15	3	101.50	4.62		
p1	11	4	123.00	4.81	4	119.80
p1	12	4	116.60	4.76		
p1	14	7	98.50	4.59	7	88.20
p1	13	7	77.90	4.36		
p1	15	8	27.20	3.30	8	26.90
p1	16	8	26.60	3.28		
p1	11	9	45.00	3.81	9	36.50
p1	12	9	28.00	3.33		
p1	13	10	23.30	3.15	10	32.55
p1	14	10	41.80	3.73		
p1	16	11	32.30	3.48	11	28.15
p1	15	11	24.00	3.18		
p1	12	15	2.89	1.06	15	5.16
p1	11	15	7.43	2.01		
p2	11	1	191.00	5.25	1	171.10
p2	12	1	151.20	5.02		
p2	13	2	134.60	4.90	2	129.25
p2	14	2	123.90	4.82		
p2	16	3	114.90	4.74	3	112.45
p2	15	3	110.00	4.70		
p2	11	4	72.50	4.28	4	72.05
p2	12	4	71.60	4.27		
p2	14	7	54.00	3.99	7	54.55
p2	14	7	55.10	4.01		
p2	15	8	21.40	3.06	8	34.30
p2	16	8	47.20	3.86		
p2	12	9	24.60	3.20	9	21.35
p2	11	9	18.10	2.90		
p2	14	10	13.90	2.63	10	17.85
p2	13	10	21.80	3.08		
p2	15	11	21.40	3.06	11	14.78
p2	16	11	8.16	2.10		
p2	12	15	1.74	0.55	15	2.42
p2	11	15	3.10	1.13		
p3	12	1	91.00	4.51	1	94.15
p3	11	1	97.30	4.58		
p3	14	2	64.60	4.17	2	66.35
p3	13	2	68.10	4.22		
p3	16	3	56.00	4.03	3	52.60
p3	15	3	49.20	3.90		
p3	11	4	33.60	3.52	4	34.10

Position	Level	Time	Conc.	Ln (conc)	Time	Avg (conc)
p3	12	4	34.60	3.54		
p3	14	7	14.30	2.66	7	15.95
p3	13	7	17.60	2.87		
p3	15	8	10.60	2.36	8	10.09
p3	16	8	9.58	2.26		
p3	11	9	7.63	2.03	9	6.75
p3	12	9	5.87	1.77		
p3	14	10	4.57	1.52	10	4.51
p3	13	10	4.45	1.49		
p3	15	11	4.22	1.44	11	3.60
p3	16	11	2.97	1.09		
p3	11	15	1.23	0.21	15	1.06
p3	12	15	0.89	-0.12		
p1	13	16	3.2			
p1	14	16	7.9			
p2	13	16	6.7			
p2	14	16	1.6			
p3	13	16	0.73			
p3	14	16	0.58			

Mice set No. 1
Date of injection 86/9/29

Regression Output:rad=300

Constant	5.905016		
Std Err of Y Est	0.373652	uCi.d=	1369.06 uCi.d
R Squared	0.911401	HL=	2.59 Days
No. of Observations	20	Dose=	293.03 rad
Degrees of Freedom	18		

X Coefficient(s) -0.26797
Std Err of Coef. 0.019693

Regression Output:rad=200

Constant	5.587775		
Std Err of Y Est	0.346753	uCi.d=	930.93 uCi.d
R Squared	0.931959	HL=	2.41 Days
No. of Observations	20	Dose=	199.25 rad
Degrees of Freedom	18		

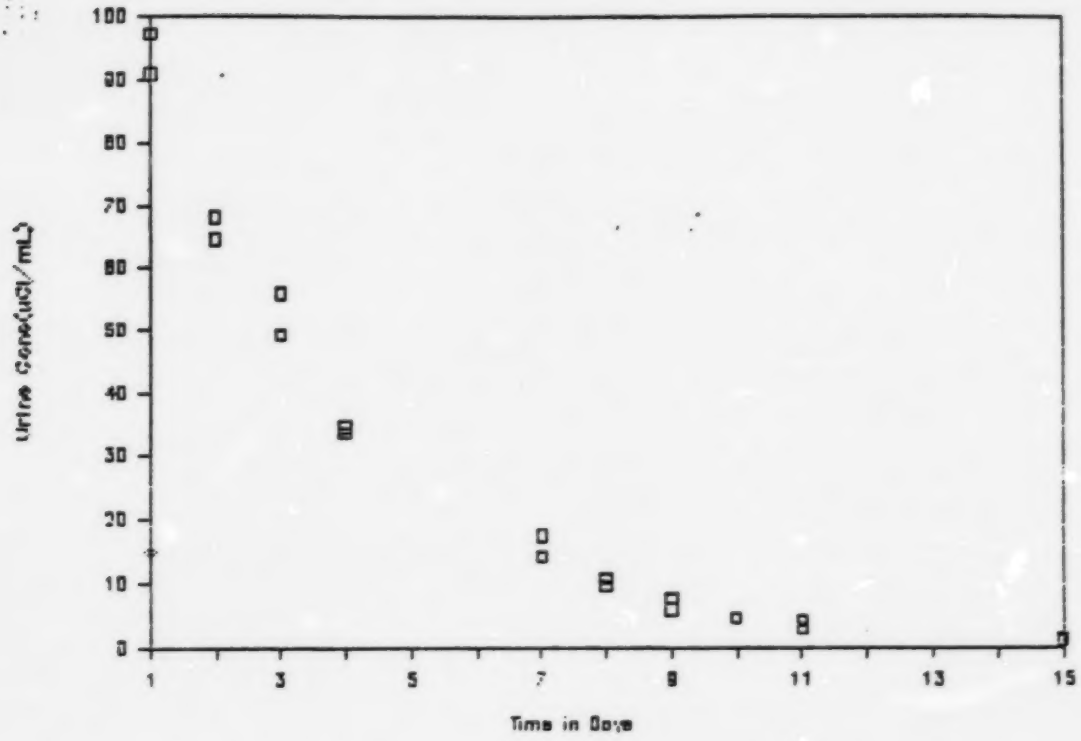
X Coefficient(s) -0.28696
Std Err of Coef. 0.018275

Regression Output:rad=100

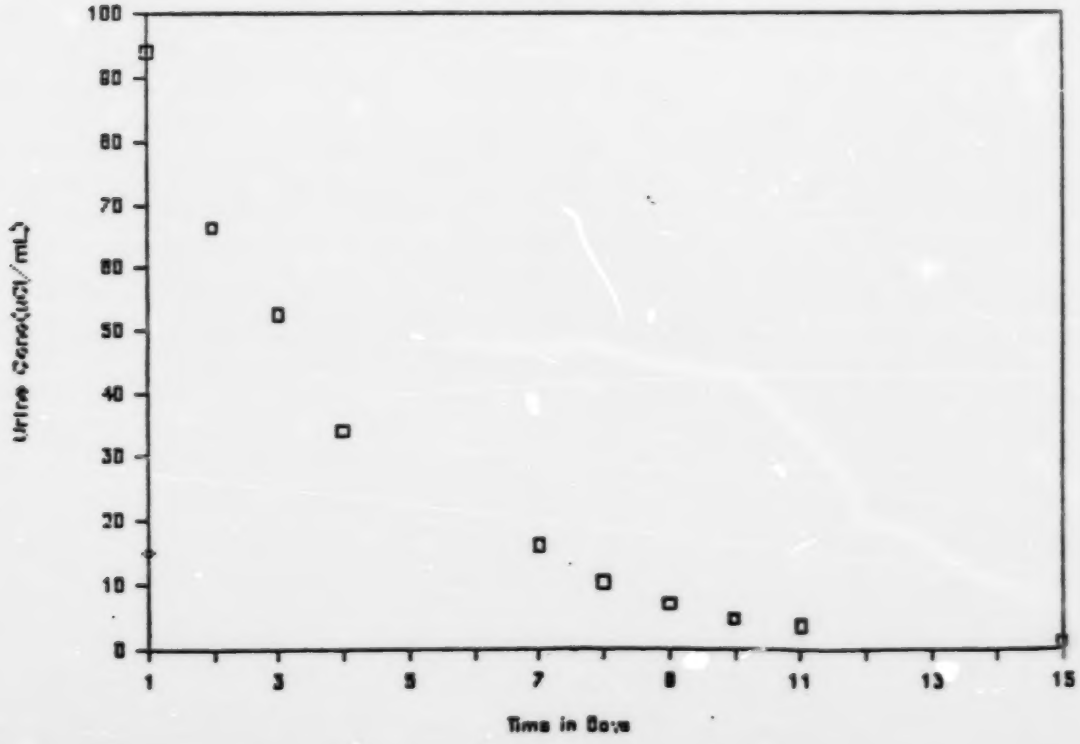
Constant	4.882495		
Std Err of Y Est	0.128504	uCi.d=	405.13 uCi.d
R Squared	0.992277	HL=	2.13 Days
No. of Observations	20	Dose=	86.71 rad
Degrees of Freedom	18		

X Coefficient(s) -0.32572
Std Err of Coef. 0.006772

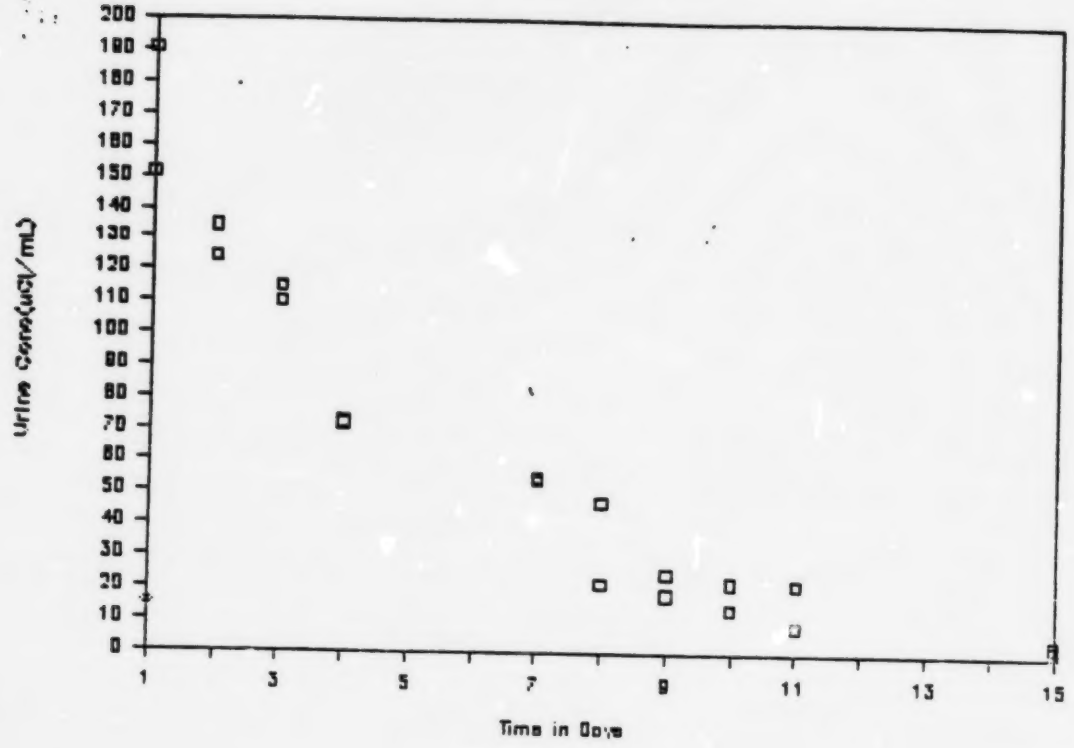
Set 1 100rad



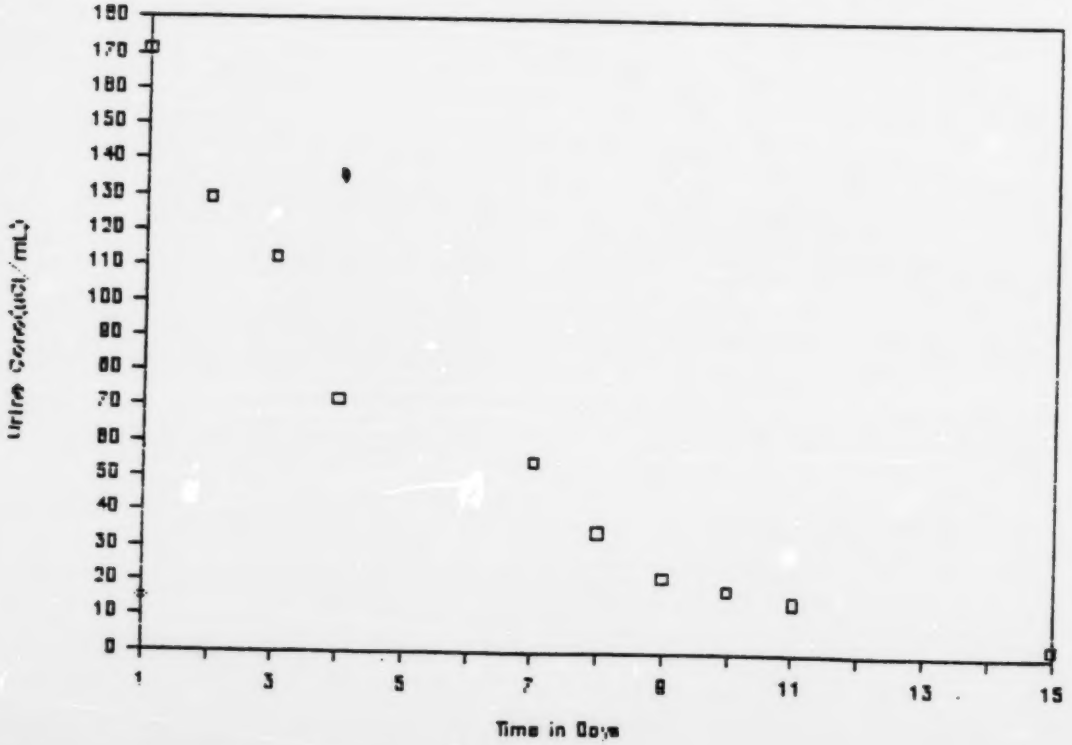
Set 1A 100rad (Average)



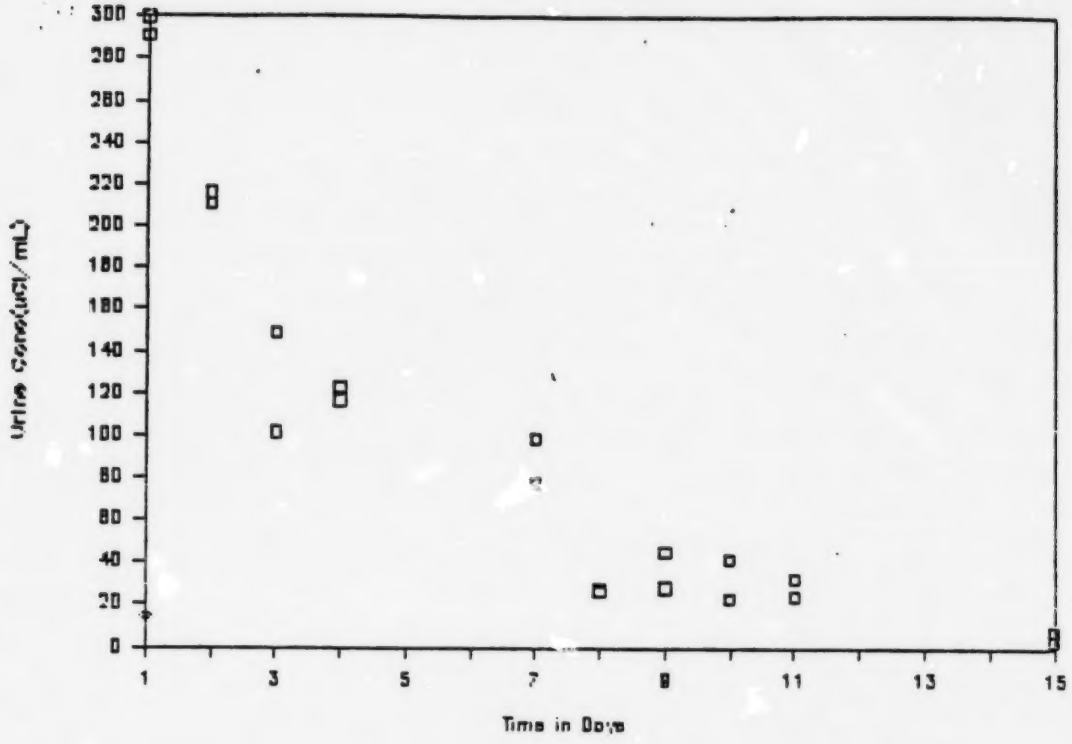
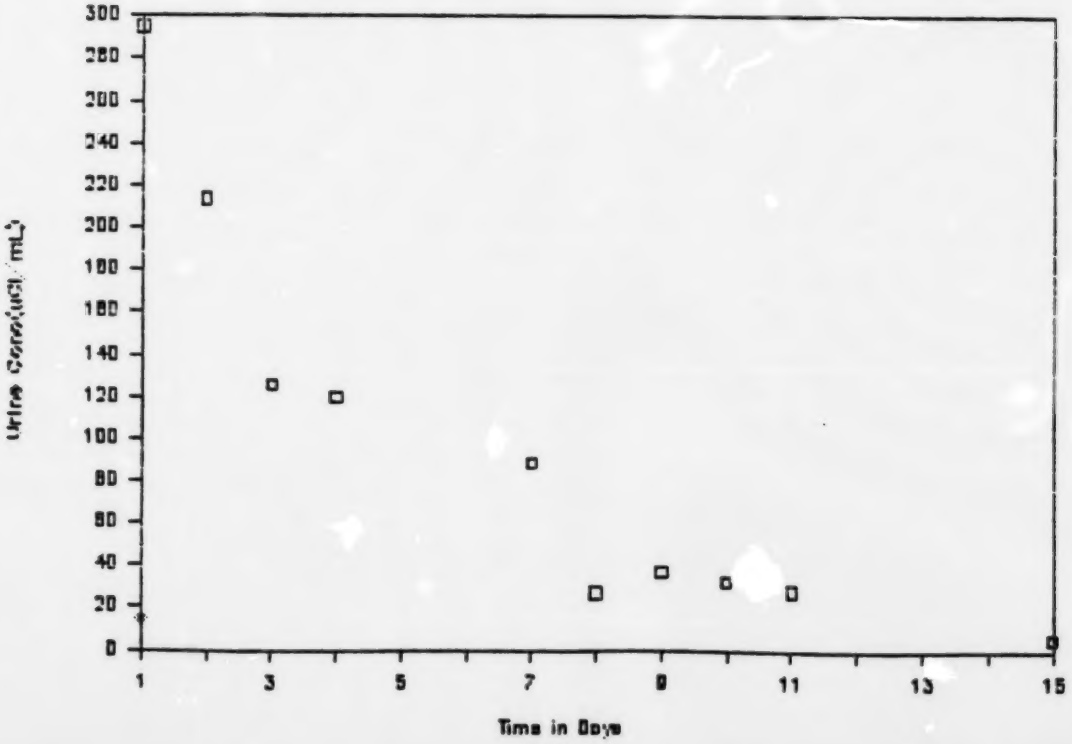
Set 1 200rad



Set 1A 200rad - (Average)



Set 1 300rod



Set 1A 300rod (Average)